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Food Safety Knowledge, Attitude, and Practices of Meat Handlers in Ghazni, Afghanistan

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ABSTRACT

Introduction: Food hygiene is vital in food safety, and meat is essential to food. On the other hand, different types of meat are consumed worldwide. In addition, food hygiene, knowledge, attitude, and practice can directly influence the quality and marketing of food. This study evaluated meat handlers' knowledge, attitude, and practices in Ghazni, Afghanistan.

Methods: This cross-sectional study was conducted on 30 meat handlers' food hygiene in Ghazni, Afghanistan. The data were collected through a face-to-face questionnaire. The respondents were selected randomly, and the data were analysed using the IBM SPSS Statistics software version 24.

Results: The majority of respondents were middle-aged, 26–35 years (43.4%), most of them were married (83.3%) and had primary education (43.3%). Most respondents did not have health certificates or participate in food safety-related training (96.7%). Most respondents generally had a high level of food safety knowledge and attitude, with a lower score in meat hygiene practices.

Conclusions: Lack of food safety and health training by meat handlers can be a risk for the consumer. Therefore, meat handler health certificates, food hygiene attitudes, and practices should be checked by governmental and non-governmental organizations for the health of consumers and better hygienic practices.

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Introduction

Food safety lets consumers know that foods do not contain toxic, chemical, or microbial contaminants and prevent these hazards from occurring in foods. In addition, food safety knowledge (FSK) is understanding food from skills or schooling, food safety attitude (FSA) refers to sensation or belief about food safety, and food safety practice (FSP) indicates the act or use of food safety (1). Food safety concerns the food industry, consumers, and regulatory agencies worldwide. Millions of people die yearly, and many are hospitalized globally from foodborne diseases and illnesses due to contaminated food consumption (2). Low- and middle-income countries are much more affected by foodborne diseases due to poor food safety training, noncompliance with hygiene practices, insufficient potable water, and unhygienic storage (3). The food handler's knowledge, attitude, and hygienic practices directly relate to food safety and security. Food safety training programs, workshops, and health certificates are essential for food handlers' working activities. The increasing food safety knowledge of meat handlers does not improve their knowledge, attitudes, and practices, but they remain essential for better performance (4).

The food processing area susceptible to food contamination and the spread of foodborne diseases is within the meat handling and slaughtering sectors. According to Nyamakwere et al. (5), the meat handling section in food processing plants is characterized by intensive handling and slaughtering of carcasses in a multi-step process. Therefore, poor hygienic practices (e.g., non-use of gloves, protective clothing, and disinfectants) in meat handling facilities can lead

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to food contamination and the spread of foodborne diseases (6).

Most food handlers in Afghanistan do not use gloves while processing food, apply poor hygienic practices in eating eggs and meat, and lack awareness of raw food eating in some cases of parasitic disease. Although most people dry meat in the fall season and use it in the winter because they cannot access electricity or refrigerators, these types of meat can also cause foodborne diseases. The cases mentioned above result from most food-borne disease outbreaks in Afghanistan. On the other hand, there are no formal and informal studies on meat handler assessment in Afghanistan to estimate their meat hygiene knowledge, attitude, and practice. Only some studies in food hygiene, safety, and security have been conducted in Afghanistan. This study aimed to estimate meat handler knowledge, attitude, and hygienic meat handling practices in Ghazni, Afghanistan.

Material and Methods

Study Area

The study was conducted in the municipal slaughterhouse of Ghazni, Afghanistan. Ghazni province is located in the southeast region of Afghanistan with a transitional climate change between semi-arid with a cold winter and a warm, dry summer (2). This cross-sectional study was conducted between Jun to September 2022. Questionnaires were used to estimate the meat handlers' food safety knowledge, attitude, and practice. All questionnaires were administered via face-to-face interviews, and their meat handling hygiene and practices were revised to ensure the precision of the respondents. The respondents were interviewed during their free working time to give enough time to answer written queries and avoid distraction from business. A total of 30 respondents were selected randomly based on the population and number of meat handlers in Ghazni, Afghanistan, who work in sheep, cattle, and chicken slaughterhouses in Ghazni.

Questionnaire Structure

The study questionnaire consisted of three parts. The first part of the questionnaire consisted of the socio-economic characteristics of the respondent based on age, gender, education level, years of experience and food safety-related training, religion, monthly income, and marital status. The second part was about the respondent's information on meat hygiene knowledge and included 20 questions on personal hygiene, the risk of carcass contamination, the importance of refrigerators, and the risk of foodborne illness to humans. The respondents had three-answer of true, false, and not sure choice key. The attitude section included 18 questions about personal protection and slaughter hygiene that participants could answer with the two-choice answer key of agreeing or not sure. The last section on meat hygiene practices had 20 questions on personal and slaughter hygienic practices. In addition, the respondent had two yes or no choice answer keys. The questionnaire was read and distributed during the interview, and meat handlers had enough time to answer the questions.

Data Analytical Technique

The data was analysed by SPSS software version 24.

Results

The results are divided into different separate sections.

Socio-economic Profile

The socio-economic profile of the respondents is shown in Figures 1. a, b, c, d, e, f, g, and h. The respondents within the age range of 26–35 years were the majority (43.3%), followed by under 25 years (33.3%), 36-45 years (16.7%), and above 45 years (6.7%). On the other hand, most of the respondents were male, and only one was female. However, the education level of the respondents (43.3%) was primary, followed by bachelors and illiteracy (23.3%). In addition, most respondents were married (83.3%) and Sunni (76.7%). Although the monthly income of the majority of respondents (56.7%) is in the range of 10000 Afghanis, which is a little more than 100 dollars per month, among the respondents, only one of them earned 31000–50000 Afghanis per month. Most respondents did not have a health certificate or participate in any food safety training (96.7%) (Figure 1).

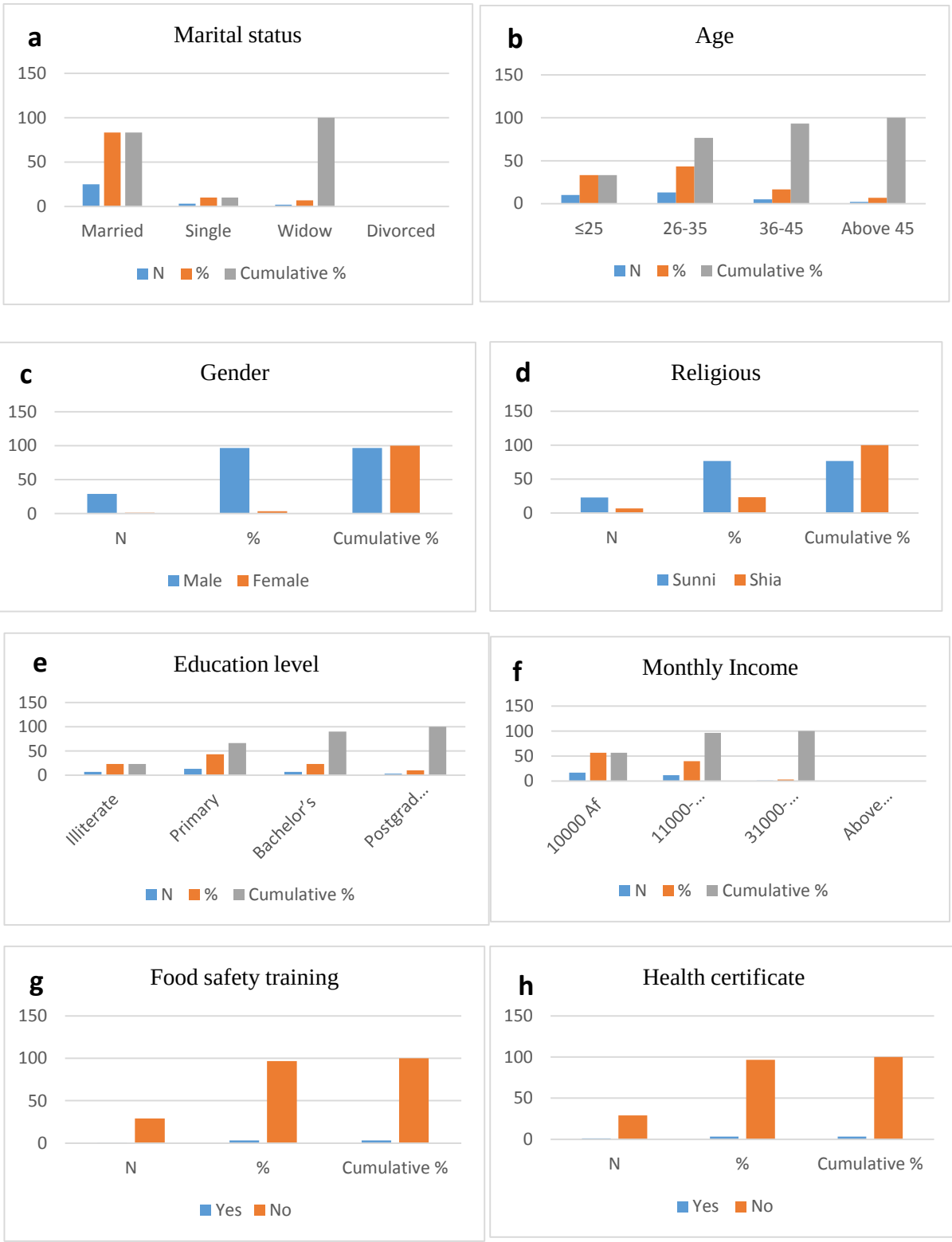

Figure 1. Meat handlers Socio-economic characteristics in Ghazni City (n = 30)
AF: means Afghani, the local currency of Afghanistan.

Food Safety Knowledge

Table 1 presents respondents' overall knowledge level about personal hygiene, causes and transmission of foodborne diseases, cross-contamination, and refrigerator uses of meat handlers in Ghazni, Afghanistan, respectively. Most respondents were assured that food safety knowledge is essential (96.7%) for meat handlers to better meat handling. Although the meat handlers were aware that insects and pests are the source of contamination (83.3%), some respondents believed that the agent of diarrhoea was transmitted by food to consumers (53.3%) (Table 1).

Table 1. Meat handlers food safety knowledge in Ghazni City (n = 30).

No	Statements	Respondents' n and %					
		True		False		Not sure	
		n	%	n	%	N	%
1	Regular hand washing during the meat processing can reduce the risk of meat contamination.	26	86.7	2	6.7	2	6.7
2	The use of gloves during meat handling can reduce the risk of meat contamination.	23	76.7	2	6.7	5	16.7
3	Meat inspection plays an important role in internalizing infection.	26	86.7	0	0.00	4	13.3
4	Refrigeration of meat is important for its preservation.	19	63.3	4	13.3	7	23.3
5	Cross-contamination from contaminated meat to meat transmitted by meat handlers.	16	53.3	7	23.3	7	23.3
6	Before slaughtering, it is important to wash the live animal.	6	20.0	19	63.3	5	16.7
7	The rotten and clean parts of the meat should be processed separately.	22	73.3	7	23.3	1	3.3
8	Knowledge about food safety is essential.	29	96.7	0	0.00	1	3.3
9	The carcass of an animal in a dirty environment causes it to rot.	28	93.2	2	6.7	0	0.00
10	Improper handling of meat can create risks for the consumer.	11	36.7	5	16.7	14	46.7
11	Improper handling of meat could pose a health hazard to consumers.	23	76.7	2	6.7	0	0.00
12	Proper cleaning and sanitization of knives and hooks can reduce the risk of meat contamination.	24	80.0	3	10.0	3	10.0
13	Eating and drinking in the workplace can increase the risk of meat contamination.	20	66.7	7	23.3	3	10.0
14	Washing and disinfection of working surfaces and tools are important for the safety of meat.	21	70.0	5	16.7	4	13.3
15	Insects and pests could be a source of raw meat contamination.	25	83.3	2	6.7	3	10.0
16	The agent of diarrhoea can be transmitted by food.	16	53.3	6	20.0	8	26.7
17	Contaminated meat always has some change in color, odor or taste.	19	63.3	6	20.0	5	16.7
18	People with open skin injuries, gastroenteritis and ear or throat diseases should not be allowed to handle meat.	21	70.0	8	26.7	1	3.3
19	The health status of a worker should be evaluated before employment.	20	66.7	6	20.0	4	13.3
20	The ideal place to store raw meat is the refrigerator.	16	53.3	11	36.7	3	10.0

Food Safety Attitudes

Table 2 shows meat handlers' attitudes, and about 78.3% of the respondents have a good attitude about food safety in Ghazni, Afghanistan. Most respondents were assured that meat hygiene training is necessary for their work, and 96.7% and 93.3% agreed that cleaning surfaces can reduce the risk of illness. However, 96.7% of the meat handlers agreed that proper handling is the job of meat handlers. In comparison, 43.3% of the respondents were uncertain that leaving meat for more than 2 hours outside the refrigerator is unsafe (Table 2).

Meat Handler Practices Of Meat Hygiene

Table 3 represents meat handlers' meat processing practices in Ghazni, Afghanistan. The respondents washed their clothes daily, and only two had a yes answer (6.7%). About 86.7% did not wash animals after slaughtering, and 93.3% did not touch meat with blood for freshness. Most respondents use water for meat processing (96.7%). There was an inspection of animals after slaughtering in 96.7% of cases, slaughtering area. Wearing a mask, washing hands after the toilet, and taking out equipment occurred when going to the toilet in 96.7 cases. The majority of the respondents failed the smoking inside meat

processing areas (76.7%), wear nail polish during meat processing of meat in the duration of illness (60%), and take out equipment when going to the toilet (96.7%).

As shown in Table 3, most of the ill meat handlers handle meat (56.7%); on the other hand, meat handlers with cuts, injuries, and bruises handle meat (66.7%). This result can impact the consumers negatively, and some gastrointestinal diseases can be transferred to consumers through meat.

Table 2. Respondents attitudes toward meat hygiene in Ghazni City (n = 30).

No	Statements	Respondents n and %					
		Agree		Uncertain		Disagree	
		N	%	n	%	N	%
1	Meat hygiene training provides the necessary material for meat handlers.	29	96.7	1	3.3	0	0.00
2	Wearing of protective clothing and shoes can improve food hygiene.	26	86.7	4	13.3	0	0.00
3	Using watches, earrings and rings will increase the risk of meat contamination.	23	76.7	2	6.7	5	16.7
4	Inspection of meat before and after slaughtering can produce healthy meat.	24	80.0	5	16.7	1	3.3
5	Regular training could improve meat safety and hygienic practices.	30	100	0	0.00	0	0.00
6	Keeping the working surfaces and utensils clean, can reduces the risk of illness.	28	93.3	2	6.7	0	0.00
7	Meat handlers containing zoonotic diseases can contaminate meat.	19	63.3	8	26.7	3	10.0
8	It is necessary to sanitize or change knives after the meat process.	24	80.0	4	13.3	2	6.7
9	Improper storage of meat is dangerous for human health.	27	90.0	3	10.0	0	0.00
10	Using different knives and cutting boards for meat and offal is assets it.	25	83.3	4	13.3	1	3.3
11	It is unsafe to leave meat out of the refrigerator for more than 2 hours.	14	46.7	13	43.3	3	10.0
12	Raw meat is healthier and more nutritious than cooked meat.	7	23.3	5	16.7	18	60.0
13	Knives, hooks and cutting boards can be the sources of meat contamination.	19	63.3	6	20.0	5	16.7
14	Sneezing or coughing without covering nose and mouth could contaminate meat.	14	46.7	3	10.0	13	43.3
15	It is important to wash working surfaces and cutting tools after disinfection.	25	83.3	2	6.7	3	10.0
16	Putting on a head covering is a good practice in meat processing.	22	73.3	6	20.0	2	6.7
17	Inspection of meat for freshness and wholesomeness is valuable.	27	90.0	3	10.0	0	0.00
18	Handling of meat in a proper way is one of the meat handler's jobs.	29	96.7	1	3.3	0	0.00

Table 3. Respondents hygienic practices of meat assessment in Ghazni City (n = 30)

No	Statements	Respondents % n			
		Yes (n %)		No (n %)	
1	Do you wash your clothes after every working day?	2	6.7	28	93.3
2	Do you process animal carcasses and by-products in the same place?	1	3.3	29	96.7
3	Do you wash your hands while working?	28	93.3	2	6.7
4	Do you use enough water for meat processing?	29	96.7	1	3.3
5	Do you wash animals before slaughtering?	4	13.3	26	86.7
6	Do you touch meat with blood after processing for freshness?	2	6.7	28	93.3
7	Do you refrigerate meat after processing?	18	60.0	12	40.0
8	Do you inspect animals before slaughtering?	29	96.7	1	3.3
9	Do you smoke inside the meat processing area?	16	53.3	14	46.7
10	Do you wear mask while working?	29	96.7	1	3.3
11	Do you wear an apron while working?	28	93.3	2	6.7
12	Do you wash your apron at the end of every working day?	23	76.7	7	23.3
13	Do you wash your hands after product processing?	25	83.3	5	16.7
14	Do you wash your hands after using the toilet?	29	96.7	1	3.3
15	Do you wash your hands after sneezing, coughing and smoking?	7	23.3	23	76.7
16	Do you wear cap or protective clothes while working?	20	66.7	10	33.3
17	Do you wear nail polish during meat handling?	12	40.0	18	60.0
18	Do you handle or process meat when you are ill?	17	56.7	13	43.3
19	Do you handle or process meat when your hand has cuts, injuries and bruises?	20	66.7	10	33.3
20	Do you take out your equipment when you go to the toilet?	29	96.7	1	3.3

Discussion

The socioeconomic results showed that most of the meat handlers were male. The results were consistent with Jianu and Goleț (6) and Kamal et al. (8), but not consistent with (7). In addition, females were not allowed to work outside the home in Afghanistan. On the other hand, the slaughtering work is very heavy and complex, the

women cannot work in slaughterhouses, but they can work in some poultry slaughterhouses, especially in rural areas (8, 9). In addition, most respondents were in the 26–35 age range because most middle-aged people in Afghanistan are responsible for preparing food and other family requirements because women and children do not work outside the home. In our study, literacy levels were higher than other findings (7, 10). In Afghanistan, many literate people are jobless because there are no work opportunities in governmental and non-governmental organizations, and they also face private working opportunities. The lack of food handler training and health certificates negatively affected their hygienic activities. Only one meat handler participated in food safety training. However, previous studies have shown that food safety training should be provided to improve food safety knowledge, attitude, and hygienic practices (9). The reasons for the development in food safety are related to food safety education and health training. On the other hand, education has many social benefits, like better hygiene and sanitation facilities, the availability of quality food, food hygiene, higher economic returns, and better access to technology and sources of information (8).

The meat handlers' food safety knowledge showed that food contamination is transmitted to the consumers. The transmission is due to the lack of food safety training offered in the study (4), which showed a higher percentage (93.41%). The respondents had a high level of knowledge in washing and cleaning, but few consumers knew about health risks and the importance of refrigeration. Most meat handlers did not use refrigerators because of a lack of electricity. Metal rings were used in front of their shops for meat for better marketing and consumer attention. According to Todd et al., most of the foodborne outbreaks globally are caused by food handlers (11). In addition, Sharif and Al-Malki reported that food handlers' knowledge, attitude, and practice play an essential role in food poisoning outbreaks (12).

According to the meat handler's food safety attitude in the current study, the respondents in Ghazni had a low percentage, and 46.7% of the respondents said that sneezing or coughing without covering their noses or mouths could contaminate the meat. This result was not in line with (13). The low attitude is also related to the lack of meat handlers' health certificates, food safety training, and formal and informal education.

According to the meat handler's food safety hygienic practices, all food safety practices were related to the economy. On the other hand, poverty is one of the leading causes for the consumption of unsafe food, attributable to lack of access to adequate food and clean water, poor arrangements in government structures, perpetuating infectious diseases in the community, unsafe environmental situations to ensure food safety, and poor food handling and sanitation practices (14). According to previous studies, Afghanistan is one of the least economically developed countries in the world. There are many problems with sanitation and other hygienic practices because all hygienic practices require consumers' awareness, a better economic situation, and day-to-day hygienic practices. Furthermore, food-borne diseases happen in the food chain, from production to consumer (Table 2). Several studies worldwide have shown that food handlers' educational status impacts food-handling practices (15, 16). Other studies have indicated that the knowledge of food handlers affects their food-handling practices (17, 18). In addition, most foodborne diseases resulted from poor meat processing by meat handlers, while meat handlers were responsible for foodborne outbreaks (4). In this study, most of the meat handlers in Ghazni had a high level of meat handling knowledge, but some of them lacked knowledge of refrigeration and improper handling of diseases of some meat. Most respondents had a high food safety attitude, meat handling, and hygienic practices. According to all past research, the respondents have positive knowledge of food safety, but low food safety practices are due to the lack of food safety training and health certificates of food handlers (17, 19, 20, 21, 22, 23).

Conclusion

Based on the results, the respondents had poor percentages in meat hygienic practice and refrigeration despite the meat handlers' high food safety knowledge and attitude. In addition, the respondents did not participate in any food safety training and did not have health certificates. Food safety training can affect their food safety and attitude positively. The present study reveals that only one food handler has a

health certificate, and most foodborne diseases are transmitted through food. The Afghanistan government should control all meat handlers due to their health certificates and other essential training and hygienic aspects.

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Conflict of Interest

The authors declare no conflict of interest.

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Improvement of the Immune System with Two Types of Emergency Rations in the Murine Animal Model

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ABSTRACT

Introduction: Rescue and preservation of refugees and disaster victims depend on delivering cost-effective, nutritionally sound food options. Utilizing food items enriched with vital nutrients and immune system fortifiers is imperative to bolster and sustain proper immune system functionality. This study explores the immunomodulatory impacts of two emergency rations on the immune system using a murine animal model.

Methods: In this study, four sets of ten Balb/c strain mice aged between 4 and 6 weeks, weighing 17.8 to 18.9 grams, were handpicked. Two of these groups were subjected to treatment diets designated as 1 and 2, while the other two groups were provided with control diets numbered 1 and 2 administered at 3 to 4 grams daily over eight weeks. Following the 8-week dietary intervention, blood samples were collected to evaluate interleukin-4 (IL-4), interferon-gamma (IFN-γ), immunoglobulin G 1 (IgG1), and IgG2 levels.

Results: The outcomes revealed that the treatment groups exhibited significantly higher IFN-γ levels than their control counterparts. Additionally, the IFN-γ/IL-4 ratio was consistently elevated within the treatment groups as opposed to the control groups. There was a significant enhancement in cellular immune responses within the treatment group, as indicated by an increase in Th1/Th2 cell ratios. Moreover, in the treatment group, there was a significant increase in IgG2 antibodies and a corresponding decrease in IgG1 antibodies compared to the control group .

Conclusions: Based on the results, using emergency rations in mice increased cellular immune responses in both treatment groups.

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Introduction

Every year, natural or human-made disasters affect large human populations. Examples of such disasters are earthquakes, floods, wildfires, hurricanes, chemical spills, nuclear plant accidents, and wars. Anxiety, stress, and severe tension resulting from disasters or emergencies often lead to a lack of appetite in affected individuals. Thus, survivors cannot use many common foods due to power outages and the destruction of warehouses and refrigeration facilities, which prevent the distribution of regular food by relief organizations. Iran is a disaster-prone country, ranking fourth in Asia and sixth globally in natural disasters [1]. Furthermore, a military crisis is not out of the question, considering its geopolitical position and the presence of external enemies.

As natural and human-induced catastrophes become more frequent, developing emergency food provisions for relief missions has become a top priority for crisis management. Specialized rations and emergency sustenance solutions have been meticulously crafted within the United States to address these pressing needs.

The emergency ration is considered in classical and guerrilla wars and military maneuvers alongside operational rations to support forces that have not supplied food for more than 24 hours [3]. Various rations, including biscuit-like

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and bar-like forms, are produced for these purposes [3,6].

In the formulation of these food products, the synthesis of five pivotal factors takes precedence: (1) paramount product safety, (2) an element of palatability, (3) streamlined distribution logistics, (4) user-friendliness, and (5) comprehensive nutrient content [5]. Emergency food products should furnish the essential energy, proteins, vitamins, minerals, and other nutrients indispensable for sustaining human life during brief periods of crisis. Preserving microbial safety, nutritional value, and oxidation resistance emerge as quintessential traits for a long-lasting, shelf-stable product under adverse environmental conditions [2]. Furthermore, the sensory attributes of these products should resonate with diverse cultural norms and preferences [7]. Thus, the harmonious amalgamation of these elements becomes pivotal in designing an ideal emergency ratio.

The composition, encompassing macronutrients and such products' sensory and physical properties, has been meticulously expounded in antecedent reports and research endeavors [2]. These rations' moisture content and water activity should hover around 9.5% and 0.6% to prevent microbial spoilage, nutrient degradation, and oxidative degradation [5]. Ideally, an emergency ration should boast a shelf life of no less than 36 months, even when stored at 21℃. Furthermore, each serving of these rations ought to yield approximately 233kcal. As a result, adults consume roughly 9 to 10 servings per day, equating to about 2100 kilocalories daily. The constitution of these rations becomes paramount, considering that these products are expected to serve as an individual's exclusive source of sustenance for up to 15 days.

A myriad of ingredient combinations have been explored across various studies to create these emergency rations, including soy products (ranging from flour and concentrate to isolate) as primary protein sources, low-fat or fat-free powdered egg and milk, semi-hydrogenated soybean oil, vegetable oils, and hydrogenated fats for the requisite fat content. Additionally, grain-based mixtures, vitamin and mineral premixes, sugar, and potentially cooking agents have been employed as complementary components [3,5]. Harmonizing these constituents is essential, allowing the resultant product to withstand diverse distribution methods, including airdropping, even under the most challenging conditions.

The characteristics of emergency food products have been studied using various formulations with different technologies. Additionally, affordability and the potential to enhance the consumer's immune system have received less attention. Stress levels during crises and wartime conditions weaken the immune system, making individuals more susceptible to diseases. Therefore, producing rations that can improve individuals' immune status during crises can provide the necessary nutrients and make them more resistant to potential illnesses. Moreover, food items such as grains, their products, and their abundant nutrients have functional properties in foods, which are readily available and cost-effective, making them suitable for various food formulations [4].

An exemplary emergency ration should encompass adequate proportions of essential components, including plant oils, proteins, carbohydrates, and vitamin/mineral premixes. The immune system's role, particularly innate and cell-mediated immunity, holds paramount importance in preventing and managing microbial infections. Incorporating foods enriched with immune-boosting nutrients becomes imperative to fortify and sustain optimal immune system performance. Nutrition deficiencies often affect the immune system, particularly cell-mediated immunity, phagocyte function, cytokine production, and antibody secretion. Malnutrition stands as a pervasive driver of immune system impairments on a global scale [13,16].

Therefore, this study aimed to unveil the immunomodulatory impacts of two distinct emergency rations through the lens of a murine animal model.

Materials and Methods

This study used a diet with formulation number 1, developed by Dehghani Moghadam and colleagues. The only change in the diet was the variation in zinc, selenium, and vitamin D levels to investigate their immunomodulatory effects. In the Dehghani Moghadam and colleagues' study, diet formulation number 1 had the highest acceptability regarding sensory characteristics, primarily composed of wheat flour and powdered milk. Furthermore, none of the tested microorganisms, including Klebsiella,

Salmonella, molds, yeast, and *E. coli*, were detected in any treatment group [8].

Composition of Treatment Diet 1

Wheat flour (25g), powdered milk (5g), canola oil (8g), sugar (7g), and, in equal proportions, lecithin (0.05g), vanilla (0.05g), cocoa powder (0.5g), coconut powder (0.75g), BHT (as an antioxidant) (0.005g), salt (0.2g), water (4 to 6mL), 0.3 tablets of Imustim containing dried extract of Echinacea purpurea (as an immune system enhancer), vitamin/mineral premix (3.5g), and maltodextrin (0.25g) were added. The micronutrients for the emergency diet are determined based on Table 1.

Table 1. Emergency feed micronutriensts for treatment group 1

Micronutrient type	The amount in each approximately 50 gr of bar
Vitamin A (In the form of capsulated palmitate)	116.55 µg
Vitamin D_3 (In the form of cholecalciferol)	1.11 µg
Vitamin E (In the form of acetate)	3.33 UI
Vitamin K_1 (In the form of phytonadione)	0.011 mg
Vitamin C (In the form of capsulated ascorbic acid)	31.10 mg
Vitamin B_1 (In the form of capsulated thiamine mononitrate)	0.19 mg
Vitamin B_2 (In the form of riboflavin)	0.20 mg
Niacin (In the form of niacinamide)	1.33 mg
Vitamin B_6 (In the form of pyridoxine hydrochloric acid)	0.22 mg
Folic Acid	0.044 mg
Vitamin B_{12} (In the form of cyanocobalamin)	2.78 µg
Biotin	5.56 µg
Pantothenic Acid (In the form of D-Calcium pantothenate)	0.78 mg
Calcium (In the form of tricalcium phosphate or calcium carbonate)	66.67 mg
Phosphorus (In the form of dipotassium phosphate or tricalcium phosphate)	111.11 mg
Magnesium (In the form of magnesium oxide)	22.2 mg
Zinc (In the form of zinc oxide or zinc sulfate)	2.06 mg
Copper (In the form of copper oxide or copper gluconate)	0.10 mg
Manganese (In the form of manganese sulfate)	0.056 mg
Selenium (In the form of sodium selenate or selenomethionine)	4.44 µg
Chromium (In the form of chromium chloride ($6H_2O$))	2.78 µg
Iodine (In the form of potassium iodide)	0.011 mg
Iron (In the form of ferric EDTA or chelated iron)	1.89 mg
Potassium (In the form of dipotassium phosphate)	204.44 mg
Choline (In the form of lecithin)	769 mg

Composition of Treatment Diet 2

Wheat flour (25g), powdered milk (5g), canola oil (8g), sugar (7g), and, in equal proportions, lecithin (0.05g), vanilla (0.05g), cocoa powder (0.5 grams), coconut powder (0.75 grams), BHT (as an antioxidant) (0.005g), salt (0.2g), water (4 to 6mL), 0.3 tablets of Imustim containing dried extract of Echinacea purpurea (as an immune system enhancer), vitamin/mineral premix (3.5g), and maltodextrin (0.25g) were added. The micronutrients for the emergency diet are determined based on Table 2, based on which This diet's vitamin D, selenium, and zinc content were increased.

Composition of Control Diet 1

The diet used for the control group was provided by Javaneh Khorasan Company and included the following components: Protein: 20-21%, Fat: 2-3%, Energy: 2750kcal/kg, Crude fiber: 5-6%, Methionine: 0.05%, Lysine: 0.05%, Salt: 0.5%, Calcium-to-phosphorus ratio: 1.5-2.5%, Ash: 4%, Soybean meal, canola meal, cottonseed meal, molasses, salt, phosphate, methionine, vitamin and mineral supplement, fish meal, and wheat bran.

Composition of Control Diet 2

Wheat flour (25g), powdered milk (5g), canola oil (8g), sugar (7g), and, in equal proportions, lecithin (0.05g), vanilla (0.05g), cocoa powder (0.5g), coconut powder (0.75g), BHT (as an antioxidant) (0.005g), salt (0.2g), water (4 to 6mL).

In pursuit of pioneering research, ten cohorts of Balb/c mice, aged between 4 and 6 weeks and exhibiting a weight range of 17.8-18.9g, were assembled. In this study, two of these groups were entrusted with treatment diets denoted as 1 and 2, while an additional two groups were provided with control diets marked as 1 and 2. These diets were diligently administered at a daily rate of 3-4g over eight weeks. The vigilant observations encompassed the mice's overall

well-being, water consumption, and food intake throughout this duration. Blood samples were harvested to meticulously gauge the concentrations of IL-4, IFN-γ, IgG1, and IgG2 after this 8-week dietary intervention, as detailed in reference [15].

Table 2. Emergency feed micronutriensts for treatment group 2

Micronutrient type	The amount in each approximately 50 gr of bar
Vitamin A (In the form of capsulated palmitate)	116.55 µg
Vitamin D_3 (In the form of cholecalciferol)	5 µg
Vitamin E (In the form of acetate)	3.33 UI
Vitamin K_1 (In the form of phytonadione)	0.011 mg
Vitamin C (In the form of capsulated ascorbic acid)	31.10 mg
Vitamin B_1 (In the form of capsulated thiamine mononitrate)	0.19 mg
Vitamin B_2 (In the form of riboflavin)	0.20 mg
Niacin (In the form of niacinamide)	1.33 mg
Vitamin B_6 (In the form of pyridoxine hydrochloric acid)	0.22 mg
Folic Acid	0.044 mg
Vitamin B_{12} (In the form of cyanocobalamin)	2.78 µg
Biotin	5.56 µg
Pantothenic Acid (In the form of D-Calcium pantothenate)	0.78 mg
Calcium (In the form of tricalcium phosphate or calcium carbonate)	66.67 mg
Phosphorus (In the form of dipotassium phosphate or tricalcium phosphate)	111.11 mg
Magnesium (In the form of magnesium oxide)	22.2 mg
Zinc (In the form of zinc oxide or zinc sulfate)	500 mg
Copper (In the form of copper oxide or copper gluconate)	0.10 mg
Manganese (In the form of manganese sulfate)	0.056 mg
Selenium (In the form of sodium selenate or selenomethionine)	2 mg
Chromium (In the form of chromium chloride ($6H_2O$))	2.78 µg
Iodine (In the form of potassium iodide)	0.011 mg
Iron (In the form of ferric EDTA or chelated iron)	1.89 mg
Potassium (In the form of dipotassium phosphate)	204.44 mg
Choline (In the form of lecithin)	769 mg

Selection of Primary Ingredients

The choice of key constituents for formulating dietary compositions was rooted in a multifaceted evaluation, encompassing nutritional excellence, local availability in Iran, economic viability, widespread cultural endorsement among the Iranian populace, and alignment with prevailing dietary inclinations. Most of the ingredients for diet production were sourced from Shahrvand chain stores in Tehran, while some were sourced from confectionery ingredient distributors in the city. Wheat flour, soy flour, powdered milk, and canola oil were considered sources of carbohydrates, protein, and fat, respectively. Lecithin, vanilla, cocoa powder, coconut powder, BHA, salt, water, and vitamin/mineral premixes were also added to the formulations. The ratios of these ingredients in the formulations are listed in Table 1. Only the antioxidant butylated hydroxyanisole (BHA), lecithin (as an emulsifier), and maltodextrin (as a bulking agent) were procured from Merck (Germany). The vitamin premix for diets 1 and 2 was formulated by Eswe Iran Pharmaceutical Company, considering the recommended amounts by the Institute of Medicine.

Diet Preparation

A progressive approach was employed for diet preparation. Initially, canola oil was introduced into a beaker and subjected to gentle heating in an oven set at 112°C, maintaining the process until complete liquefaction ensued. Simultaneously, the dry constituents of each formulation underwent meticulous blending for 5 minutes, utilizing a mixer of Tefal makes, hailing from France. Subsequently, lecithin was judiciously incorporated into the molten oil, ensuring thorough dissolution. This amalgamation was introduced into the preceding mixture with 5 minutes of rigorous mixing. Water was added at the end of the process to facilitate the creation of the definitive dough-like substance, which required another five minutes of comprehensive blending. This resultant amalgam was adeptly poured onto aluminum foil and sculpted to dimensions measuring 7.6 ×4.4 cm. During baking, the molds were carefully placed in an oven set at 150°C for exactly 20 minutes. Following baking, the diets were carefully enclosed in polyethylene packaging and meticulously stored at 38°C, awaiting subsequent experiments, as detailed in reference [8].

Modification of Diets for Immunomodulation

Iron was included as iron oxide or sulfate to transform the diets into immunomodulatory agents with immune-enhancing properties and infection resistance, and selenium and vitamin D were added to each tablet or dough. Only in diet two the levels of iron, selenium, and vitamin D were increased. These diets were available to the mice for an 8-week, during which no other food except water was provided (Tables 1 and 2).

ELISA Procedure

Dilution of Standard Solutions

The standard solutions were diluted uniformly in 5.1mL microtubes, following the kit instructions. The ELISA procedure, including the addition of blood serum samples, was carried out as follows:

1. **Control Wells**: These wells served as blanks and received only a combination of chromogen solution A, B, and the stop solution.
2. **Standard Solution Wells**: 50µL of standard solution and 50µL of streptavidin-HRP were meticulously introduced into these wells.
3. **Sample Wells**: The sample wells initiated with the addition of 40µL of blood serum samples, followed by the sequential introduction of 10µL of interleukin-4, interferon-gamma, IgG1, IgG2 antibodies, and 50µL of HRP-streptavidin. Subsequently, the plate was securely covered, gently agitated, and incubated at 37°C for 60 minutes.
4. **Wash Solution Preparation**: A potent wash solution (30X) was expertly crafted by judiciously diluting it with distilled water, paving the way for upcoming crucial steps.
5. **Thorough Washing**: The plate cover was adroitly removed, and the liquid contents were judiciously discarded from each well. Subsequently, each well was diligently flooded with the prepared wash solution. The solution was swiftly drained after a brief 30-second interval. This crucial step was repeated five times to ensure thorough washing, followed by careful blotting of the plate until drying.
6. **Chromogenic Reaction**: The plate was gently agitated to foster a harmonious blend of the contents, commencing with the introduction of 50µL of chromogen solution A, followed by 50µL of chromogen solution B into each well. The plate then embarked on a controlled incubation journey, held at a consistent temperature of 37°C for precisely 10 minutes, shrouded in darkness to facilitate optimal color development.
7. **Reaction Termination**: About 50µL of the stop solution was systematically introduced into every well with precision. This judicious addition transformed from the initial blue hue to a resplendent yellow.
8. **Assessment**: This pivotal phase was conducted with meticulous timing, within a strict 10-minute window post-addition of the stop solution. The absorbance (OD) measurement for each well was exactingly executed at a wavelength of 450nm, employing a state-of-the-art ELISA reader (EL Bioteck, 800X). The blank served well as the reference point, assigning it a zero value for accurate comparisons.

Based on the standards and the optical density (OD) readings of the samples, the concentrations of the factors were calculated in pg/mL [9].

Statistical Analysis

Microsoft Excel software (Macintosh version 2016) was harnessed as the analytical tool of choice to analyze all the data gathered, encompassing sensory analysis scores and microbial counts of the samples. A rigorous statistical evaluation was undertaken to assess potential disparities between treatment-related mean results, employing a one-tailed, one-way analysis of variance (ANOVA) methodology executed using SPSS IBM software (version 24). A significance threshold of 5% ($P<0.05$) was diligently adhered to throughout the analytical process.

Results

The outcomes pertaining to alterations in interleukin-4, gamma interferon, IgG1, and IgG2a concentrations are elucidated as follows:

In interleukin-4, the treatment group exhibited a notably lesser increment than the control group. A significant difference was observed in the concentration of this cytokine regarding treatment group 1 in comparison to both control group 1 ($P=0.001$) and control group 2 ($P=0.003$), as well as in treatment group 2 compared to control group 1 ($P=0.001$) and control group 2 ($P=0.018$).

Conversely, the level of gamma interferon in the treatment group displayed a significantly more pronounced elevation when contrasted with the control group. Significant concentration shifts were established in treatment group 1 about control group 1 ($P=0.012$) and control group 2 ($P=0.03$), as well as in treatment group 2

compared to control group 1 (P=0.001) and control group 2 (P=0.018).

On a divergent note, the concentration of IgG1 exhibited a reduction within the treatment group as opposed to the control group. Significant differences in concentration were observed in treatment group 1 compared to control group 1 (P=0.002) and control group 2 (P=0.03), as well as in treatment group 2 in contrast to control group 1 (P=0.006) and control group 2 (P=0.01).

Furthermore, the level of IgG2a within the treatment group demonstrated a marked escalation when juxtaposed with the control group. Concentration fluctuations of statistical significance were discerned in treatment group 1 when compared to control group 1 (P=0.014) and control group 2 (P=0.02), as well as in treatment group 2 vis-à-vis control group 1 (P=0.001) and control group 2 (P=0.04) (Table 3).

Table 3. The results of changes in the concentration (pg / mL) of blood IL4, IF Gamma, IgG1.IgG2 (standard deviation±mean) at different times before and after eating two practical emergency food rations in the treatment group and before and after eating two normal food rations in control group

		Time	
Measured factors	**Group**	**Before receiving the emergency food ration**	**8 weeks after receiving food ration**
IL4 (Pg/ml)	Treatment 1	1.2830±..23712	42.35±#10.7*×
	Treatment 2	1.2820±23701	46.88±4.83 #*×
	Control 1	1.2848±.24712	78.3509±9.97
	Control 2	1.3348±.28708	65.18±11.67
IF Gamma (Pg/ml)	Treatment 1	152.10±26.06	4.92±# 32.67*
	Treatment 2	150.30±23.01	-30.97±×#46.44*
	Control 1	149.40±25.01	24.27±47.15
	Control 2	152.31±27.20	25.96±65.69
IgG1 (Pg/ml)	Treatment 1	1.2848±.24	.396±.646 *×#
	Treatment 2	1.1938±.18	.476±# .264*×
	Control 1	1.2520±.26	1.28±.24
	Control 2	1.2638±.25	.831 ±.493
IgG2a (Pg/ml)	Treatment 1	152.10±26.06	#-188.3±9.61*
	Treatment 2	150.19±19.9	171.21±17.87
	Control 1	148.34±30.03	132.1026.06
	Control 2	149.07±24.04	126.70±35.01

*: Significant changes compared to before receiving food ration in the same group.
#: Significant changes compared to the control group 1 in 8 weeks after receiving food ration
×: Significant changes compared to the control group 2 in 8 weeks after receiving food ration

Discussion

Iran, a country susceptible to various disasters, is frequently hit by substantial financial and human losses caused by natural calamities. A staggering 31 of the 40 recognized categories of natural disasters worldwide occur in Iran. Furthermore, the prospect of military crises looms ominously, given Iran's pivotal strategic and geopolitical positioning, coupled with the persistent specter of external threats. Emergency diets often emerge as the sole lifeline in these dire scenarios, providing sustenance in the early throes of catastrophes like earthquakes, hurricanes, and the difficulties of war zone evacuations. Within this context, emergency diets' nutritional potency, sensory appeal, and immune system fortification offer paramount significance in meeting the acute dietary needs of those thrust into these harrowing circumstances [10,11].

Mice consuming the treatment diets exhibited elevated levels of gamma interferon compared to their counterparts in the control groups. Moreover, the IFNγ/IL4 ratio within the treatment groups exceeded that within the control groups, associated with an increase in the Th1/Th2 cell ratio and a stronger cellular immune response within the cohort. Accordingly, the treatment group showed an increase in IgG2 antibodies and a decrease in IgG1 antibodies compared to the control group. This shift in IgG2 and IgG1 isotypes among the mice is underpinned by the influence of IFNγ and IL4, aligning seamlessly with the cytokine analysis results, thereby substantiating the heightened

Th1 cell ratio in the treatment-receiving group [15].

Protein malnutrition, a profound concern, exerts significant deleterious effects on cellular immune response, phagocytic function, complement system activity, secretory immunoglobulin A antibody concentrations, and cytokine production. Even relatively mild nutrient deficiencies can lead to tangible alterations in immune responses. Among the crucial micronutrients, zinc, selenium, iron, copper, and vitamins A, C, E, B, and folic acid profoundly influence immune responses [15].

Alberts et al. 2003 delved into the immunomodulatory potentials of dietary supplements incorporating vitamins A, E, C, selenium, and zinc using a mouse model. Researchers investigated phagocytic activity, oxidative responses, gamma interferon, interleukin 4, and immunoglobulin G after sensitizing mice with dinitrochlorobenzene. In this study, dietary vitamin A supplementation induced heightened inflammatory responses decreased Th1 responses, and increased mucosal responses. Young mice who received insufficient nourishment in the form of vitamin C, E, selenium, or zinc exhibited no discernible impact on their immune systems, which resonates with the results from the present study [12].

Kiremidjian et al. (1990) examined the effects of selenium-containing dietary supplements in mice for eight weeks and investigated the effects of these supplements on interleukin 1 and 2. In this study, a selenium diet significantly affected lymphocyte proliferation. Furthermore, selenium supplementation increased interleukin levels considerably. Therefore, mechanisms responsible for the effects of immune responses through lymphocyte proliferation are independent of IL-2 or IL-1 levels [14].

Ramiro-Puig et al. (2007) explored the influence of cocoa-rich diets on the intestinal immune system of desert mice. Over three weeks, these intrepid mice were nourished with cocoa-rich diets, and their immune system dynamics underwent thorough scrutiny to assess critical factors such as immunoglobulin A, interleukins 2, 4, 10, and gamma interferon. Astonishingly, the findings unveiled a transformative impact that consumption of cocoa-rich diets culminated in the proliferation of mesenteric lymph nodes and amplification of Peyer's patches. Furthermore, the T-cell ratio and antigen receptor activity within both lymphatic tissues was augmented. This groundbreaking study unequivocally validated that cocoa-rich diets served as catalysts for heightened production of immunoglobulin A and gamma interferon while concurrently suppressing interleukin ten levels. In essence, cocoa consumption was shown to profoundly influence the modulation of the intestinal immune response, particularly in young mice [23].

These intriguing revelations serve as poignant reminders of nutrition's pivotal role in fortifying immune responses, a factor of paramount significance, especially in the difficulties of emergencies and disasters. Specifically, tailored diets enriched with specific nutrients can profoundly impact immune system performance. Further exploration and in-depth research in this field promise to unveil novel avenues to enhance immunity and resilience in diverse crises and disasters.

Conclusion

Based on the results, considering all aspects, including sensory evaluation, microbial tests, total fat percentage, fat oxidation levels, water activity, and production economics, the three-year stability at a temperature of 21°C and, most importantly, the enhanced immune response in the mouse model, these diets can be regarded as an acceptable regimen for boosting the immune system for use in emergency conditions in the country. Therefore, further investigation and validation in humans are required. Compared with foreign emergency diets, this diet costs half to one-third less, and bulk ingredient procurement can make it even cheaper.

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Probiotic Consumption, Fatigue, and Glycemic Control in Patients with Type 2 Diabetes: A Cross-Sectional Study

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ABSTRACT

Introduction: Probiotics have recently been included in nutritional recommendations for achieving glycemic control in diabetic patients. Probiotic foods are not standardized, and their effectiveness can vary significantly between products and species. Therefore, the results of this study may not be generalizable to all probiotics consumed. This study aimed to determine the consumption of probiotics by type 2 diabetes patients and the relationship between probiotic consumption and their fatigue levels and glycemic control.

Methods: This study was conducted in a university hospital in the Central Anatolian Region of Turkey. A total of 235 diabetic patients were included in the cross-sectional study. Data were collected using a patient information form, a self-report probiotic consumption information form, and the Visual Analog Scale for Fatigue.

Results: The majority of the patients (83.4%) consumed probiotic products, and the most frequently consumed probiotic products by them were yogurt (80%), ayran (67.7%), and pickles (57.9%). The fatigue levels of probiotic-consuming and non-consuming patients were similar ($p > 0.05$), but the energy levels of probiotic-consuming patients were higher ($p<0.05$). The fasting blood glucose and HbA1c levels of the patients taking probiotics were low, but this difference was insignificant ($p>0.05$).

Conclusion: Since probiotics are beneficial to diabetes patients, it is essential to provide information about them and support the use of probiotics per expert recommendations.

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Introduction

The prevalence of type 2 diabetes is increasing worldwide, and it is a complex disease influenced by genetics and the environment (1). A lack of effective management of type 2 diabetes can lead to both short- and long-term complications (2). In recent years, the importance of gut microbiota has been examined to prevent the development of type 2 diabetes, and much attention has been drawn to the consumption of probiotics in maintaining a healthy state (3).

Probiotics contain living microorganisms called friendly or good bacteria that benefit health when taken in sufficient quantities (1,4). Probiotics have high numbers of microorganisms with no pathogenic or toxic properties. Probiotics are resistant to food additives and processing conditions, maintain their viability in foods during storage and usage, and keep their vitality in the intestines and metabolic activity in the body. In addition, probiotics can colonize the gastrointestinal tract by attaching to the intestinal epithelium and inhibit the attachment of pathogenic bacteria to the host by secreting antimicrobial substances (5). In addition to regulating intestinal flora (6, 7), they have many health benefits, including immune regulation and inflammatory functions and the production of short-chain fatty acids by the fermentation of dietary fiber. Probiotics regulate the secretion of glucose and fat metabolism, modulate intestinal permeability and intestinal hormones, increase absorption of minerals, and improve gastrointestinal functions (8,9). Additionally, probiotics can enhance the antioxidant defense, regulate blood glucose by improving insulin sensitivity and pancreatic β-cell processes, balance the blood lipid profile, and control weight, especially in diabetic patients (1,3). Meta-analyses have shown that probiotic consumption

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facilitates diabetes management and reduces glycosylated hemoglobin (HbA1c), fasting blood glucose (FBG), and insulin resistance (10-13).

Probiotics have been consumed for centuries in different cultures through different foods (14). A wide range of milk and products, including fermented milk, yogurt, and kefir, as well as capsules and powders, contain these nutrients (1). Additionally, some fermented herbal products, pickles, cabbage, turnip, boza, kumiss, fermented meat, soy-based products, cereals, nuts, fruits, legumes, and various non-dairy fruit juices are also among probiotic food items (15,16). Consumption of probiotics has recently gained significant attention because of reducing markers of oxidative stress, inflammatory factors, and metabolic parameters (17). Probiotics also have a variety of other benefits, such as increasing glutathione (GSH) levels, scavenging hydroxyl and superoxide radicals, and reducing interleukin-6 (IL6) production. There is little information about how probiotics affect metabolic control in individuals with diabetes. However, studies have mainly been conducted on animal models and non-diabetic patients (18). Yadav et al. showed that lactobacillus acidophilus and L. casei containing high fructose-fed rats reduced glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative oxidative stress (19). In another study, consumption of L. plantarum (299v) lowered systolic blood pressure, serum insulin levels, leptin, fibrinogen, F2-isoprostanes, and IL-6 (20).

There have been no studies showing a superiority between probiotics in different amounts and products, to our knowledge (21).

Fatigue is one of the most common symptoms that develop due to physiological and psychological causes in diabetic patients (22), which is a persistent and disturbing complaint in patients with type 2 diabetes. In addition, fatigue can adversely affect the well-being of patients with diabetes, their activities of daily living, family, work, and social lives (23). Physiopathological changes occur at the cellular level due to long-term hyperglycemia in diabetes caused by poor metabolic control. The first clinical sign of these changes is the emergence of fatigue symptoms with a severe decrease in patients' exercise capacity (20-24). Many causes of fatigue can be observed in patients with diabetes, including episodes of hypoglycemia, difficulties in self-care, complications of diabetes, endocrinopathy, and infection (25). Therefore, controlling hyperglycemia in diabetic patients can effectively alleviate fatigue (22). At the same time, it was emphasized that probiotics can be an alternative treatment for chronic fatigue (26).

Literature review showed that studies examine the relationship between probiotic consumption and glycemic indicators in type 2 diabetes patients (27-33), but the relationship between probiotic consumption and fatigue levels has not been examined. Probiotics, which support the immune system, may have fatigue-reducing properties. Probiotic use and fatigue may contribute to science by guiding healthcare professionals in dealing with fatigue, a common symptom of type 2 diabetes.

Methods

Objective and Design

This cross-sectional study was conducted to determine the consumption of probiotics by diabetic patients and the relationship between the probiotic consumption of these patients and their fatigue levels and glycemic control status.

Population and Sample

The study population consisted of 1220 individuals who presented to the endocrinology outpatient clinic of a university hospital between January and June 2022 and had been diagnosed with diabetes for at least one year. The minimum number of patients to be included in the sample was found to be 215 using the sampling formula for a known population (Nt^2pq / ($d^2(N-1)$ + t^2pq)). In this context, 235 patients who met the inclusion criteria were included. The inclusion criteria were being literate, 18 years old and above, having type 2 diabetes, being independent in self-care, and agreeing to participate in the study. The exclusion criteria included having a verbal communication disorder, being diagnosed with an eating disorder, being pregnant, taking regular probiotic supplements, using insulin, cholesterol, or diuretic drugs, and having a gastrointestinal disease (such as Crohn's disease ulcerative colitis).

Data Collection Tools

The data were collected using a patient information form, a self-report probiotic consumption information form, and the Visual Analog Scale for Fatigue (VAS-F).

Patient Information Form: The form consisted of three parts with questions on the

sociodemographic characteristics of the patients (age, sex, marital status, education, employment), disease characteristics (e.g., duration of disease, type of treatment, regular use of drugs) in the second section, and metabolic parameters (e.g., FBG, HbA1C, blood pressure, total cholesterol, triglyceride).

Probiotic Consumption Information Form: The form was created to determine the probiotic consumption characteristics of the patients and contains questions on parameters such as whether the patients knew about probiotics, the types of probiotics they consumed, the frequency of their consumption, and the amount of their consumption. As a pilot implementation, the form was administered to 20 individuals with diabetes before starting the study and evaluated in terms of intelligibility.

Visual Analog Scale for Fatigue: VAS-F was developed by Lee et al. to measure fatigue and energy levels. The Turkish validity and reliability study of the scale was carried out by Yurtsever and Beduk (34,35). The fatigue and energy dimensions of this scale are composed of 18 items. VAS-F consists of 10 cm-long horizontal lines with positive statements at one end and negative statements at the other. Fatigue items progress from positive to negative, while energy items progress in the opposite direction. The lowest and highest scores in the fatigue dimension are 0 and 130. There is a 0 to 50 score range for the energy dimension. High scores in the fatigue dimension and low in the energy dimension indicate higher fatigue severity (31-35). In this study, Cronbach's alpha internal consistency coefficient of the scale was found to be 0.86 for the fatigue dimension and 0.93 for the energy dimension.

Data Collection

The researchers obtained the data by interviewing patients in an allocated interview room. Researchers informed the patients verbally about the study and administered the data collection forms to those who consented verbally and in writing. Approximately 20 minutes were spent applying the data collection forms to each patient. Additionally, the metabolic parameters of the patients, including routine follow-up results, were obtained from their laboratory result papers after the physician ordered the measurements at the time of their visit to the outpatient clinic.

Table 1. Disease-Related Characteristics of Patients

Characteristics	n	%
Disease duration (years) (Mean±SD)	8.06±5.52	
Form of treatment		
Oral antidiabetic therapy	148	63.0
Insulin therapy	48	20.4
Oral antidiabetic and insulin therapy	39	16.6
Treatment is administered regularly		
Yes	141	60.0
Sometimes	66	28.1
No	28	11.9
Does regular exercise (at least three times a week and for at least 20 minutes)		
Yes	201	89.3
No	24	10.7
Has received education about the disease from a doctor or nurse		
Yes	140	59.6
No	95	40.4
Has other chronic diseases		
Yes	113	48.1
No	122	51.9
Has diabetes-related complications		
Yes	53	22.6
No	182	77.4
Frequency of hospitalization due to diabetes or complications in the past year		
Once	146	62.1
Twice	59	25.1
Three or more times	30	12.8
General health assessment		
Good	65	27.7
Medium	152	64.7
Bad	18	7.6

Data Analyses

The data were analyzed using SPSS software version 22.0. Students' t-tests were used in the analysis of the data in addition to descriptive statistical methods to determine the relationship between probiotic consumption, metabolic control, and fatigue levels. Furthermore, multiple linear regression analysis was used to determine the explanatory effect of some variables for fatigue. Statistical significance was evaluated at a threshold of $p<0.05$.

Ethical Aspects of the Study

Before starting the study, written permission was obtained from the Sivas Cumhuriyet University Non-Invasive Clinical Research Ethics Committee (Decision No: 2020-02/10) and the institution where the study was conducted. Additionally, the purpose of the study was explained to diabetic patients, and written and verbal consent was obtained from the patients who agreed to participate. The patients were informed that the data they would provide would only be used within the scope of the study, and their confidentiality would be ensured.

Table 2. Distribution of Metabolic Parameters of Patients

Characteristics	Mean ± SD	n	%
Fasting blood glucose (mg/dl)	168.90 ± 98.50		
<100		21	8.9
≥100		214	91.1
HbA1C (%)	7.35 ± 1.51		
<7.0		119	50.6
≥7.0		116	49.4
Systolic blood pressure (mmHg)	127.18 ± 17.92		
≤140		214	91.1
>140		21	8.9
Diastolic blood pressure (mmHg)	75.91 ± 10.14		
≤80		206	87.7
>80		29	12.3
Total cholesterol (mg/dl)	216.71 ± 59.14		
<200		72	30.6
≥200		163	69.4
Low-density lipoprotein (mg/dl)	188.36 ± 102.68		
<100		168	71.5
≥100		67	28.5
High-density lipoprotein (mg/dl)	56.71 ± 22.09		
>40 in men, >50 in women		24	1.7
<40 in men, <50 in women		231	98.3
Triglyceride (mg/dl)	199.85 ± 78.42		
<150		64	27.2
≥150		171	72.8

Results

The mean age of the diabetes patients participating in the study was 52.87±11.85 years, and 84.7% of them were under 65 years of age. While 53.2% of the patients were male, 80% were married, 33.6% were primary school graduates, 62.6% did not work in any job, and 8.9% lived alone. Only 10.7% of the patients exercised regularly. About 27.2% of the patients were current smokers, and 5.1% consumed alcoholic beverages. Moreover, 42.1% of the patients were overweight, and 37.4% were obese. Table 1 shows the disease-related characteristics of the patients.

The metabolic parameters of the patients are shown in Table 2, and it was determined that 37.9% of them had glycemic control above the target value.

According to self-reports, 19.6% of the patients had two meals per day, and 35.3% had four or more meals per day. Additionally, 28.9% said they skipped meals often, while 54.5% said they occasionally. Further, 70.9% of the patients mostly skipped lunch. Nutritional supplements other than drugs were used by 6.4% of the

patients to treat diabetes. The frequently stated supplements included vitamin D, vitamin B12, and vitamin C supplements. Furthermore, 18.7% of the patients reported consuming less than one liter of fluids per day, and 50.6% stated that they consumed one or two liters of fluids. Table 3 shows some information on the probiotic consumption characteristics of the patients.

Table 3. Probiotic Consumption Characteristics of Patients

Characteristics	n	%
Knows about probiotics		
Yes	109	46.4
No	126	53.6
Consumes probiotics		
Yes	196	83.4
No	39	16.6
Type of probiotic food consumed *		
Yogurt	188	80.0
Ayran	159	67.7
Pickles	136	57.9
Olives	78	33.2
Kefir	52	22.1
Other fermented dairy products	43	18.3
Turnip	36	15.3
Tarhana	35	14.9
Goat cheese	23	9.8
Boza	3	1.3
Probiotic consumption frequency		
Once a day	117	49.8
Two to three times a day	63	26.8
Once a week	51	21.7
Rarely	4	1.7
Quantity of probiotics consumed at one time		
Half a bowl	57	24.3
A bowl	178	75.7
Feels benefit in diabetes-related symptoms related to probiotic consumption		
Yes	97	41.3
No	138	58.7
Feels benefits of probiotic in health conditions such as...		
Constipation	119	50.6
Diarrhea	21	8.8
Inflammatory bowel disease	16	6.8
Hyperlipidemia	16	6.8

* Multiple choices were allowed.

The patients' mean VAS-F fatigue dimension score was 65.16±17.37, while their mean VAS-F energy dimension score was 25.71±10.74. According to these scores, the patients had moderate levels of overall fatigue.

There was no significant difference between the fatigue levels of patients who consumed probiotics and those who did not consume probiotics ($p>0.05$), but there was a statistically significant difference between energy levels ($p<0.05$). Accordingly, A higher energy level was observed in patients who consumed probiotics.

The fasting blood glucose and HbA1c levels of the patients who were taking probiotics were low. Still, the difference between the HbA1c values of patients who consumed probiotics and those who did not was insignificant. Similarly, the metabolic indicators of the patients in these two groups did not differ significantly ($p>0.05$) (Table 4).

The multiple regression analysis determined that HbA1c was a determinant of the patient's fatigue levels, and age was a determinant of their energy levels ($p<0.05$). However, probiotic consumption

status and the frequency of probiotic consumption were not variables that were significantly associated with fatigue or energy levels (p>0.05) (Table 5).

Table 4. Comparison of Fatigue Levels and Metabolic Parameters of Patients Taking and Not Taking Probiotics

	Using probiotics (n=196; %83.4)	Don't using probiotics (n=39; %16.6)	Test
	Mean ± SD	Mean ± SD	
Visual Analogue Scale for Fatigue			
Fatigue	64.85 ± 17.24	66.71 ± 18.15	t = -0.612 *P*= .541
Energy	26.41 ± 10.83	22.23±9.66	t = 2.239 *P* = .026*
Metabolic parameters			
Fasting blood glucose (mg/dl)	160.92 ± 77.26	209.00 ± 164.76	t = -2.824 *P* = .005**
HbA1C (%)	7.30 ± 1.43	7.59 ± 1.86	t = -1.104 *P* = .271
Systolic blood pressure (mmHg)	127.32 ± 18.12	126.46 ± 17.10	t = 0.275 *P* = .784
Diastolic blood pressure (mmHg)	76.32 ± 9.91	73.84 ± 11.14	t = 1.397 *P* = .164
Total cholesterol (mg/dl)	214.90 ± 60.82	225.84 ± 49.53	t = -1.055 *P* = .292
Low-density lipoprotein (mg/dl)	56.84 ± 23.74	56.07 ± 10.55	t = 0.198 *P* = .843
High-density lipoprotein (mg/dl)	187.70 ± 104.17	191.66 ± 96.09	t = -0.219 *P* = .827
Triglyceride (mg/dl)	202.35 ± 79.97	187.30 ± 69.68	t = 1.095 *P* = .275

* *P* < .05; ** *P* < .01

Table 5. Stepwise Multiple Regression Analysis of Predictors of Fatigue and Energy Levels

Variables	Fatigue					Energy				
	B	SE	*ß*	t	*P*	B	SE	*ß*	t	*P*
Year	0.098	0.103	0.067	0.949	.344	-0.186	0.063	-0.205	-2.979	.003**
Disease duration	0.078	0.217	0.025	0.361	.718	0.039	0.131	0.020	0.297	.766
HbA1C	1.641	0.753	0.143	2.179	.030*	-0.439	0.457	-0.062	-0.962	.337
Using probiotics	0.488	3.245	0.010	0.150	.881	-2.226	1.968	-0.077	-1.131	.259
Frequency of using probiotics	0.230	1.307	0.012	0.176	.861	-0.780	0.793	-0.066	-0.984	.326
	R = 0.164, R^2 = 0.027, F = 1.259, *P* = .283					R = 0.253, R^2 = 0.064, F = 3.124, *P* = .010*				

* *P* < .05; ** *P* < .01

Discussion

Today, the consumption of nutritional supplements has increased in parallel with the awareness of healthy nutrition (15). Probiotics have a significant role in the general health of individuals and can be used as anti-diabetic agents (30). In this study, the consumption of probiotics in patients with type 2 diabetes was examined, and its effects on the fatigue levels and glycemic control statuses of these patients were evaluated.

Considering the beneficial effects of probiotics on health, individuals need to know about probiotics and their consumption (15). In this study, only about half of the patients knew about probiotics. In other studies conducted on adults in Turkey, the rates of participants who knew about probiotics varied between 46.8 and 66.5% (36-38). The low level of knowledge about probiotics and the lack of necessary guidance by doctors and/or dietitians can be cited as the reasons for the low consumption of probiotics and probiotic-added food/food supplements among the patients participating in this study (16). In a study conducted with hospitalized patients in the US, 43% of the participants stated that they knew the term probiotic (39). The result of this study, which was similar to those in the relevant literature, showed that patients with type 2 diabetes do not have sufficient knowledge about the use of probiotics. In this context, it may be

useful to inform patients with type 2 diabetes about including probiotics in their diet.

Diabetics should include whole grain products and legumes since they are low in glycemic index and regulate blood glucose more effectively than yogurt and milk (40). These foods with probiotic properties increase satiety and reduce hunger (41). The results of this study demonstrated that the majority of the patients consumed probiotics, and they mostly consumed yogurt, ayran, and pickles. Another study on diabetic patients showed that the most frequently consumed probiotic foods were yogurt, olives, and ayran. Moreover, the same study revealed that goat cheese, kefir, boza, tarhana, and pickles were the least frequently consumed probiotic foods (16). An analysis of hospitalized patients found that yogurt and cereals were the most commonly consumed probiotic products (39). Food consumption in Turkey varies according to geographical and cultural characteristics. The fact that this study was conducted in the Central Anatolian Region of Turkey may have influenced the patients' food preferences. It may be essential to create a nutrition program to support the desirable use of probiotic products in individuals with type 2 diabetes and teach this program to patients.

This study determined that the fasting blood glucose and HbA1c levels of the patients who were consuming probiotics were low. However, the HbA1c levels of the patients who consumed probiotics and those who did not were not significantly different. The literature shows that the FBG and HbA1c values of groups taking probiotics are reduced considerably compared to the control groups in randomized controlled studies (27-33). According to a meta-analysis, probiotics reduced FBG to a greater extent in the placebo/no intervention group, with a mean difference of 12.99 mg/dl in the short term and 2.99 mg/dl in the long term (13). Another meta-analysis study showed that the consumption of probiotics can reduce HbA1c, FBG, and insulin resistance in patients with type 2 diabetes (12). Similar to the findings of this study, no significant relationship was found between the consumption of probiotic foods and HbA1c in diabetic patients in a study conducted in Turkey (16). This finding, inconsistent with the literature, may have resulted from the number of different probiotics consumed by the patients and the frequency of their consumption. Probiotics may significantly impact glucose regulation when studies are conducted based on the amount of probiotics consumed.

Since probiotics interact with intestinal bacteria when digested, they positively affect physical and psychological health. Probiotics reduce cortisol, also known as the stress hormone, and increase the secretion of oxytocin, which is closely related to positive physical and psychological effects in humans (42). Fatigue levels of the patients who were consuming probiotics and those who were not did not differ significantly. On the other hand, the energy levels of patients consuming probiotics were higher. However, the consumption of probiotics and frequency did not significantly affect fatigue and energy levels. There are no similar studies conducted with diabetic patients in the literature. The information in the literature highlights that the consumption of probiotics positively affects chronic fatigue patients (43). A systematic review of probiotic consumption in athletes emphasized that probiotics improved the immune system and exercise performance, regulated immunomodulation, and reduced fatigue (14). A randomized controlled study on the effects of probiotics on mood found a significant decrease in the dimensions of unhappiness, irritability, and fatigue in the intervention group (44). This study showed that consuming probiotics helped type 2 diabetes patients feel energetic. In this context, probiotic products can be added to the diet programs of patients with type 2 diabetes, in line with the recommendations of dieticians.

Limitations

This is the only study examining the relationships between probiotic consumption in type 2 diabetes patients in Turkey and the fatigue levels and glycemic control statuses of these patients. Additionally, the results offer a different perspective on ensuring glycemic control in parallel with the increasing incidence of diabetes. However, this study had some limitations. The most important limitation of the study was that it was conducted with diabetic patients who presented to one institution at a particular time. Therefore, the findings cannot be generalized. The second limitation of the study was that the information collected on probiotic consumption and fatigue levels was based on the self-reports of the patients who took part in the study. Another limitation of the study was that the

relationship between the variables of probiotic consumption fatigue and glycemic control was only examined due to its cross-sectional design. Additionally, evaluations were made based on the amounts of the probiotic products consumed by the patients based on their self-reports, and the exact quantities of the products they consumed were not evaluated within the scope of the study. Longitudinal studies can provide more information on statistical relationships among these variables.

Conclusion

Based on the results, the majority of patients with diabetes consumed probiotic products. Although the energy levels of the patients taking probiotics were higher, glycemic control status and fatigue levels did not differ between the patients' consuming probiotics and those not consuming probiotics. There was no statistically significant difference in fatigue severity levels between the probiotic-consuming and non-consuming patients, but the finding that probiotic consumption was associated with lower fatigue severity levels suggests that probiotics may have benefits. As part of nutrition education, probiotics can be discussed with patients, and their consumption can be encouraged under expert recommendations. Furthermore, long-term case-control studies examining the impact of probiotics on fatigue levels in diabetic patients will shed light on the literature and diabetes management.

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Effects of Gamma Irradiation on Microbial, Chemical, and Organoleptic Characteristics of Ostrich Meat during Refrigeration

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ABSTRACT

Nowadays, there is a growing need to explore methods for increasing the shelf life of food. In the food industry, severe food security industrial techniques are employed, including canning, pasteurization, smoking, salting, freezing, heating, vacuum sealing, the use of chemical materials, and irradiation. This study focuses on the effects of gamma irradiation on changes in the chemical, biological, and organoleptic properties of ostrich meat. Fifteen male ostriches, aged between 10 and 14 months, underwent evaluation. Initially, the ostriches were slaughtered, and their meat) from thigh (was subjected to different irradiation doses (0, 2, 4, 6 KGY) at intervals of0, 5, 10, and 15 days. The various meat groups were then stored at 4°C.In this study, ostrich meat samples were divided into two groups: one group received no irradiation (0 kg) and the other received irradiation at doses of2, 4, and 6 kg. These samples were then stored in a refrigerator for15 days, and microbial, chemical, and organoleptic tests were conducted. The results of our investigation indicate that the 4 kg irradiation dose effectively reduced the counts of mesophilic bacteria, coliform bacteria, Staphylococcus aureus, and psychrophilic bacteria, while also eliminating Salmonella spp and E. coli spp. Additionally, it led to a reduction in Total Volatile Nitrogen (TVN) and prevented adverse organoleptic changes, including alterations in odor and color, over the 15-day refrigerated storage period. The irradiated groups also demonstrated a remarkable reduction and elimination of Staphylococcus aureus*, E. coli spp*, and *Salmonella spp* bacteria during refrigerated storage, with significant differences from the control group. Additionally, Total Volatile Nitrogen (TVN) in the control group exhibited a significant increase onthe15th day compared to the other groups. To sum up, irradiation proves to be a viable method for preserving various foods, especially meats like ostrich, and is highly recommended to safeguard against food spoilage and contamination.

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Introduction

Ostrich meat is characterized by its low cholesterol content and high levels of unsaturated fatty acids, making it a rich source of iron. Furthermore, ostrich meat does not carry the same health risks associated with other red meats like beef and lamb, which can harbor dangerous diseases that may affect human consumers.(1,2). As a result, ostrich meat is a suitable choice for a variety of individuals, including heart patients, athletes, pregnant women, children, and the elderly.(3,4)

Ostrich meat, classified as a type of red meat(5) , emerges as a compelling alternative. Beyond its protein content, ostrich meat boasts distinct attributes. It stands out with its low cholesterol levels and high unsaturated fatty acids, while also being rich in iron. Significantly, ostrich meat is devoid of the health concerns commonly associated with other red meats(6,7).

In contemporary times, the preservation of food has become imperative, given the modern lifestyle's reliance on processed and shelf-stable food resources. While the preservation of food is essential, it should not compromise the nutritional integrity of these food supplies. Within the food safety industry, an array of methods exists, encompassing canning, pasteurization, smoking, salting, freezing,

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heating, vacuum sealing, chemical additives, and irradiation.(8,9,10)

Among these, food irradiation has been introduced as a long-term preservation technique, boasting benefits such as reduced chemical usage, enhanced safety, and the significant reduction of microbial loads(11,12). Numerous studies have confirmed the technique's safety in terms of toxicology and nutritional analyses, indicating minimal changes to essential nutrients. Notably, proteins, carbohydrates, and fats remain largely unaffected, as do vital nutrients like calcium, and potassium(13,14,15).

The utilization of food radiation, including gamma rays, X-rays, or electrons, has become recognized as an effective method for pathogen eradication and the prevention of their reproduction. Among these radiation types, gamma radiation, emitted by nuclei of elements like Cs137 and Co60, holds particular importance in food preservation. It distinguishes itself through its high penetrating power, offering superior results compared to beta rays(16,17,18).

This study aims to explore the impact of gamma irradiation on ostrich meat, focusing on changes in microbial bacteria levels *(e.g., coliform spp, E. colispp, Staphylococcus aureus spp, Salmonellaspp)*, psychrophilic microorganisms, the total count of aerobic mesophilic bacteria, and chemical parameters such as total volatile nitrogen (TVN). Additionally, the study will assess the organoleptic characteristics of irradiated ostrich meat.

Materials & Methods

Sampling

This study was conducted at the Golbarg Tuba farm located in Saveh province, Iran. Fifteen ostriches, all male and aged between 10 to 14 months, were slaughtered in a slaughter house. lateral thigh muscle samples were collected using a sterile scalpel. Subsequently, ostrich meat samples were divided into two groups: one group received no irradiation (0 KGY) and the other received irradiation at doses of 2,4, and 6 KGY (each sample was divided into 16 foil-wrapped portions) and stored at refrigerator temperature. Samples were sent alongside ice to the Nuclear Agricultural Research Institute, where they were irradiated under doses of 0, 2, 4, and 6 KGY at intervals of 0, 5, 10, and 15 days. The different groups were then stored at 4°C, and their organoleptic, chemical, and microbial properties were evaluated on these respective days.

Microbial Indices

In this experiment, microbial indices were assessed, including the *Total Bacterial Count*, *Coliform* count, *E.coli* and *Salmonella* identification, *Staphylococcus aureus*, and *Psychrophilic bacteria* enumeration. *Nutrient agar*(NA) media were employed for the ***Total Bacterial Count***, **(37°C/1-2days)** (19), while *Violet Red Bile Agar* (VRBA) medium was used for the cultivation of ***Total Coliform*** (20). Also standard media containing *Brilliant Green Bile (2%) Lactose Broth*(BGB) was used for enumeration by Most Probable Number(MPN) method **(37°C/1-2days)** (21). For diagnosing E. coli, indole testing using Peptone Water and BGB was conducted, employing Kovac's reagents (44°C/1-2 days) (23). The identification and enumeration of ***Staphylococcus aureus*** were carried out using *Baird Parker Agar* medium and a 1% solution of potassium telluride**(37°C/1-2 days)**. The detection of ***Psychrophilic bacteria*** was performed using *King Agar* medium **(1-4°C/7-10days)(**24).For detection of ***Salmonella*** used *Lactose Broth*,*Selinate*,*Salmonella-Shigella Agar*,*Triple Sugar Agar* ,*Lysine Iron Agar* and *Urea* cultures ,respectively. **(37°C/1-2days)**

Total Volatile Nitrogen (TVN)

The assessment of TVN was conducted within the groups (25). In each experimental unit, 10 grams of ostrich meat were chopped and mixed with 50 ml of distilled water. Subsequently, 2 grams of magnesium oxide were transferred to a flask containing 250 ml of distilled water. A receiving dish contained 25 ml of boric acid (2%) and a few drops of tochirol reagents. The distillation flask and its contents were heated to boiling for 10 minutes. The distillation process continued for 25 minutes, and the distilled solution was finally titrated with normal hydrochloric acid (0.1N). Using the equation provided, the amount in milligrams was calculated, with each cm^3 of normal hydrochloric acid (0.1N) being equivalent to 1.4 mg TVN.

Organoleptic Tests

To assess the color and smell of the samples, organoleptic tests were conducted after exposure and at 0, 5, 10, and 15 days of storage in the refrigerator. Color was judged by five judges under natural light, each with normal eyesight,

and smell was evaluated using a cooking test. Five grams of meat samples were boiled on a direct flame in an Erlenmeyer flask containing distilled water, and the smell was assessed. Color measurements were performed prior to smell measurements. Scores for organoleptic factors were calculated, with three score categories including 'excellent,' 'good,' and 'poor' corresponding to grades of 2, 1, and 0, respectively (26).

Statistical Analysis

For the average comparison of *Total Bacterial Count*, *Coliforms spp*, *Psychrotrophic bacteria*, *Staphylococcus aureus*, and TVN in the experimental units, a two-way ANOVA was used with a 95% confidence level. Organoleptic characteristics were statistically compared using a non-parametric test (Friedman). Data concerning *E. coli and Salmonella spp*bacteria were analyzed using a chi-square test.

Results

Bacterial Counting and Identification

The results revealed a significant decrease in microbial load in all irradiated groups, with noteworthy differences among these groups. Additionally, there was a significant reduction in the *Total Bacterial Count* over the course of 0, 5, 10, and 15 days in all irradiated groups, as compared to the control group (0 KGY), which displayed statistically significant differences ($P < 0.05$) (Table 1).

Table 1. Changes in total count of bacteria (Total count), coliforms and psychrophilic bacteria (Mean log 10 cfu / g ± SE) according to different levels of radiation in the storage time of refrigerated

Changes in total count of bacteria (Total count)				
Storage time (Day)	**Dose of gamma radiation (KGY)**			
	0	**2**	**4**	**6**
0	3±36.36$^{\#}$	44.12±0$^{*\#}$	0±6.02$^{*\#}$	0$^{*\#}$
5	4±17.39$^{\#}$	1±90.17$^{*\#}$	0±32.9$^{*\#}$	0$^{*\#}$
10	5±55.38$^{\#}$	3±12.22$^{*\#}$	1±44.18$^{*\#}$	0±17.06$^{*\#}$
15	6±98.28$^{\#}$	4±12.23$^{*\#}$	2±12.16$^{*\#}$	0±76.22$^{*\#}$
Change the number of coliforms				
Storage time (Day)	**Dose of gamma radiation (KGY)**			
	0	**2**	**4**	**6**
0	0±67.15$^{\#}$	0$^{*\#}$	0$^{\#}$	0$^{\#}$
5	1±63.18$^{\#}$	0±5.04$^{*\#}$	0$^{\#}$	0$^{\#}$
10	2±60.16$^{\#}$	0±40.11$^{*\#}$	0±4.04$^{\#}$	0$^{\#}$
15	3.2±62$^{\#}$	1±46.19$^{*\#}$	0±29.09$^{\#}$	0±4.03$^{\#}$
psychrophilic bacteria count				
Storage time (Day)	**Dose of gamma radiation (KGY)**			
	0	**2**	**4**	**6**
0	0±92.11$^{\#}$	0$^{*\#}$	0$^{\#}$	0$^{\#}$
5	1±93.15$^{\#}$	0±24.05$^{*\#}$	0$^{\#}$	0$^{\#}$
10	2±85.13$^{\#}$	0±81.11$^{*\#}$	0±6.03$^{\#}$	0$^{\#}$
15	3±91.17$^{\#}$	1±54.16$^{*\#}$	0±54.09$^{\#}$	0±16.05$^{\#}$
Changes in the number of Staphylococcus aureus				
Storage time (Day)	**Dose of gamma radiation (KGY)**			
	0	**2**	**4**	**6**
0	0±16.11$^{\#}$	0$^{*\#}$	0$^{\#}$	0$^{\#}$
5	1±51.14$^{\#}$	0±5.01$^{*\#}$	0$^{\#}$	0$^{\#}$
10	2±44.15$^{\#}$	0±46.06$^{*\#}$	0±2.01$^{\#}$	0$^{\#}$
15	3±72.13$^{\#}$	1±25.10$^{*\#}$	0±31.08$^{\#}$	0±3.01$^{\#}$

$^{\#}p < 0.05$; The changes that are significantly in comparison to the group of control During different days

$^{*}p < 0.05$; The changes that are significantly in comparison to the group of control to different Gamma irradiation doses

The count of *Coliform spp* bacteria in all irradiated groups experienced a reduction over 5, 10, and 15 days. This reduction was statistically significant when compared to the control group (0 KGY). However, no significant difference was observed between the 4 KGY and

KGY groups. Meanwhile, the control group demonstrated a significant increase in the count of *Total coliforms* ($P < 0.05$) during refrigerated storage over 0, 5, 10, and 15 days (Table 1)

The *Psychrophilic* bacterial load in all irradiated groups exhibited a significant decrease when compared to the control group during refrigerated storage on days 0, 5, 10, and 15. However, there was no significant differentiation observed between the 4 KGY and 6 KGY groups. In contrast, *Psychrophilic* bacteria significantly increased in the control groups stored in the refrigerator ($P < 0.05$) (Table 1).

Also.the results indicated a reduction in *Staphylococcus aureus* in the irradiated groups during refrigerator storage on days 0, 5, 10, and 15, as compared to the control group. There was no significant difference in *S. aureus* reduction between the 4 KGY and 6 KGY groups. However, this bacterium significantly increased in different irradiated groups during refrigerated storage ($P < 0.05$) (Table 1).

The irradiated groups demonstrated a significant reduction and elimination of *E.coli spp* and *Salmonella spp* bacteria during refrigerator storage on days 0, 5, 10, and 15, in comparison to the control group ($P < 0.05$) (Table 2).

Table 2. The presence of *E.Coli* bacteria at ostrich meat according to different doses of radiation over a Storage time of refrigerated

***E.Coli* bacteria**								
Storage time (Day)	**Dose of gamma radiation (KGY)**							
	0		**2**		**4**		**6**	
	+	-	+	-	+	-	+	-
0	$9^{\#}$	6	$1^{\#}$	14	$0^{\#}$	15	$0^{\#}$	15
5	$9^{\#}$	6	$0^{\#}$	15	$0^{\#}$	15	$0^{\#}$	15
10	$9^{\#}$	6	$0^{\#}$	15	$0^{\#}$	15	$0^{\#}$	15
15	$9^{\#}$	6	$0^{\#}$	15	$0^{\#}$	15	$0^{\#}$	15
the presence of Salmonella								
Storage time (Day)	**Dose of gamma radiation (KGY)**							
	0		**2**		**4**		**6**	
0	+	-	+	-	+	-	+	-
5	$6^{\#}$	9	$1^{\#}$	14	$6^{\#}$	9	$1^{\#}$	14
10	$6^{\#}$	9	$0^{\#}$	15	$6^{\#}$	9	$0^{\#}$	15
15	$6^{\#}$	9	$0^{\#}$	15	$6^{\#}$	9	$0^{\#}$	15
0	$6^{\#}$	9	$0^{\#}$	15	$6^{\#}$	9	$0^{\#}$	15

$^{\#}p < 0.05$; The changes that are significantly in comparison to the group of control During different days

Total Volatile Nitrogen (TVN) Evaluation

On the 15th day, the control group exhibited a significant increase in TVN compared to the other groups. In contrast, the application of irradiation at doses of 2, 4, and 6 KGY led to a significant decrease in TVN in comparison with the control group (Table 3).

Table 3. TVN changes at radiation levels at ostrich meat according to storage time of refrigerated

Storage time (Day)	**Dose of gamma radiation (KGY)**			
	0	**2**	**4**	**6**
0	26.2±48.01	$23.1±13.30^{*}$	$21±10.55^{*}$	$21.1±83.22^{*}$
5	32.2±92.73	$22.1±60.22^{*}$	$24±30.95^{*}$	$23±53.91^{*}$
10	$54.4±73.23^{\#}$	$28.1±44.91^{*\#}$	$23±2.7^{*\#}$	$23.1±48.33^{*\#}$
15	$68.6±46.63^{\#}$	$36.5±64.30^{*\#}$	$2±25.11^{*\#}$	$22±87.99^{*\#}$

$^{\#}p < 0.05$; The changes that are significantly in comparison to the group of control During different days

$^{*}p < 0.05$; The changes that are significantly in comparison to the group of control to different Gamma irradiation doses

Organoleptic Tests

The color of both irradiated and non-irradiated ostrich meat samples showed a significant decrease during the storage period in the refrigerator (0, 5, 10, and 15 days). However, it is noteworthy that irradiation did not produce a significant difference in meat color when compared to the non-irradiated group (control) ($P<0.05$) (Table 4) .

Also, the smell in all experimental groups during refrigerated storage (0, 5, 10, and 15 days) was significantly reduced. These results suggested that irradiation had no significant effect on the smell of the meat ($P < 0.05$) (Table 4).

Table 4. Ostrich meat organoleptic indicators (color and smell) evaluation based on various quantities of radiation in the storage time of refrigerated

meat color				
Storage time	**Dose of gamma radiation (KGY)**			
(Day)	**0**	**2**	**4**	**6**
0	2±98.02#	2±76.04#	2±64.08#	2±68.04#
5	2±48.10#	2±33.10#	2±30.08#	2±21.11#
10	2±3.13#	1±88.16#	1±88.12#	0±2.08#
15	1±25.11#	1±70.11#	1±90.12#	1±90.17#
smell of ostrich meat				
Storage time	**Dose of gamma radiation (kilo Gray)**			
(Day)	**0**	**2**	**4**	**6**
0	2±96.03#	2±79.05#	2±66.04#	2±62.06#
5	2±46.07#	2±28.08#	2±3.09#	2±14.12#
10	1±92.06#	2±11.12#	1±88.11#	1±86.14#
15	1±4.11#	1±60.12#	1±90.10#	1±86.12#

#p < 0.05; The changes that are significantly in comparison to the group of control During different days

*p < 0.05; The changes that are significantly in comparison to the group of control to different Gamma irradiation doses

Discussion

This study highlights the potential of gamma-ray irradiation, often referred to as cold pasteurization, to reduce hazardous microorganisms in food while minimally affecting sensory and nutritional properties (27). It's important to note that while irradiation damages most microorganisms, it doesn't necessarily eliminate all of them. Hence, complementary methods like refrigeration and cooking should accompany irradiation to ensure optimal food safety (28). Combining irradiation with refrigeration has shown more substantial benefits for food safety and health compared to irradiation alone (29).

In this study, the control group showed a significant increase in *Coliform* bacteria at 0, 5, 10, and 15 days. Irradiation had no significant effect on reducing *Coliforms spp* in the control group compared to all irradiated groups. However, between the irradiated groups of 2, 4, and 6 KGY, a statistically significant difference was observed. It appears that the 4 KGY dose is particularly effective in reducing *Coliform* bacteria.Various irradiation doses (2 KGY, 4 KGY, and 6 KGY) had a significant impact on reducing the growth of *Salmonella* and *E. coli* in the present study. This aligns with Viana CM's findings in 1993, where doses between 3-5 KGY led to the inactivation of non-spore-forming bacteria in various meat types (33).

Total Bacterial counts increased significantly during storage at refrigerator temperatures on days 0, 5, 10, and 15 in different groups. This underscores the ability of irradiation to reduce bacterial counts, aligning with Mahrour et al.'s findings in 2003, which demonstrated that *Total Bacterial Counts* decreased with increasing radiation doses (34). Other studies, including that of Williams, RM in 2003, found that irradiated meats had significantly fewer bacteria compared to their non-irradiated counterparts (35). In some instances, irradiation effectively reduced *Total Bacterial Counts* in beef at doses of 1, 2, and 3 KGY (28).

Although the benefits of irradiation for various meats have been documented, limited research has explored its impact on ostrich meat shelf life. One study published in 2012 found that air-packaged ostrich meat irradiated at 1.0 KGY remained acceptable under refrigerated storage for 9 days, in contrast to 7 and 5 days for non-irradiated and samples irradiated at 3.0 KGY, respectively (18).

Food irradiation is a technology known for controlling spoilage bacteria and reducing foodborne pathogens such as *Salmonella* (36). The application of irradiation by doses of 1-3 KGY has been shown to be remarkably effective in reducing the presence of foodborne pathogens like *Salmonella*, *Toxoplasma*, *Cryptosporidium*, *Listeria*, and *E. coli* in meat, poultry, and fish (37). For instance, Thayer et al. in 1997 reported that doses of 1.5 and 3 KGY at 5°C effectively reduced various bacteria types in ostrich meat, including *Salmonella* and *Staphylococcus aureus* (30), aligning with the results of the present study. Similarly, Javanmard et al. in 2005 found that irradiation doses of 0.75, 3, and 5 KGY resulted in decreased *Total Bacterial Counts* in frozen chicken meat, with the 5 KGY dose effectively halting the growth of bacteria such as *Salmonella*

spp, *E. coli spp*, *Coliforms spp*, and *Total Bacterial Counts* during nine months (38).

Numerous studies have highlighted the effectiveness of gamma irradiation in eliminating harmful microorganisms in various food products. For instance, frozen poultry carcasses treated with 2.5 KGY irradiation have shown efficacy in destroying *Salmonella* (41, 42). D values, a measure of the radiation dose required to inactivate specific bacterial species, have been determined for different types of Salmonella. For instance, *Salmonella typhimurium* is effectively inactivated with a 0.5 KGY dose (43), while *Salmonella enteritidis* in chicken meat requires a dose of 0.37 KGY (44). In some studies, it was observed that a 6 KGY dose under refrigeration conditions prevented the growth of *Salmonella typhimurium* for up to 28 days (45). *E. coli* has also been found to be effectively inactivated after 4 KGY irradiation in fish extracts (33).

D values for *E. coli* $O_{157}H_7$ have been established, with studies indicating that doses as low as 0.27 KGY at -5°C and 0.42 KGY at 5°C are effective in eliminating this bacterium (9, 30). The sensitivity of various bacteria to irradiation has been studied, with *Campylobacter jejuni*, for example, being particularly sensitive to low-dose irradiation, effectively destroyed at a 1 KGY dose (43). In another study, it was reported that using 2 KGY of radiation reduced *Total Bacterial Counts* by 3 logs, while 4 KGY reduced counts by 6 logs, and no *E. coli* growth was observed at 8 KGY (45).

In our study, the mean logarithmic transformation of *Staphylococcus aureus* and *Psychrophilic bacteria* increased significantly in the control group during storage at 0, 5, 10, and 15 days. Irradiation with doses of 2, 4, and 6 KGY significantly reduced the numbers of *Staphylococcus aureus* and *Psychrophilic bacteria* compared to the control group. The difference between the 2 and 4 KGY irradiated groups versus the 4 and 6 kGy groups was statistically significant, consistent with other research findings.

Studies have proposed various doses of irradiation for specific bacteria. Kilinger et al. (1986) suggested a 5.4 KGY dose for reducing *Salmonella* by 2 logs and a 7 KGY dose for decontaminating poultry carcasses with *Staphylococcus aureus* and *coliforms* (30). Farkas, J (1998) indicated that irradiation at a dose of 7.2 KGY reduced foodborne bacterial pathogens, including *Salmonella*, *Staphylococcus aureus*, *Campylobacter*, *Listeria monocytogenes*, and *E. coli* $O_{157}H_7$ (46). According to the World Health Organization's technical report in 1999, relatively resistant bacteria, including *Staphylococcus aureus*, could withstand doses of 0.0-4.8 KGY irradiation, along with various species of *Salmonella*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Moraxella phenylpyruvia* growing forms (28).

Spoto et al. (2000) showed that a 6 KGY dose inhibited the growth of *Staphylococcus aureus*, *E. col sppi*, and *Salmonella typhimurium* in chicken under refrigeration conditions for up to 28 days (45). Some bacteria have been found to be highly sensitive to low-dose irradiation. For instance, Molins, RA et al. (2001) showed that bacteria such as *Yersinia SPP*, *Campylobacter SPP*, *Arcobacter butzleri*, *Pseudomonas Spp*, *Aeromonus SPP, E. coli* $O_{157}H_7$, and *Bacillus cereus* growing forms were most sensitive to 0.2 KGY irradiation (37).

Additionally, irradiation can lead to the production of volatile compounds responsible for changes in odor. For example, dimethyl trisulfide has been identified as one of the strongest-smelling compounds in irradiated raw chicken meat (49). Some studies have reported fresh and "bloody" smells in irradiated chicken and meat after cooking (50).

In our study, *Total Volatile Nitrogen* (TVN) in ostrich meat showed an increase during refrigerated storage on days 0, 5, 10, and 15 in the control group. Although the difference was statistically significant only between the 10th and 15th days compared to the first day, each irradiated group showed a significant decrease in TVN compared to the control group. While there were no statistical differences between each radiation dose, TVN analysis is a routine method for evaluating meat product quality. Irradiation can induce changes in food, both directly and indirectly, which may result in alterations in flavor and odor. Refrigeration is one of the most effective methods to reduce unfavorable flavor changes induced by irradiation. Some studies have reported popcorn or barbecue taste and smell in irradiated turkey meat, which was not present in non-irradiated samples (51).

Stephan (1998) reported that no off-flavor was observed in irradiated cooked chicken at doses below 3 KGY (52). In our present study, organoleptic tests, specifically assessing color and odor, were conducted at various time points

during the 15-day refrigerated storage period following irradiation. The results did not reveal any significant differences in color or odor. Notably, no noticeable changes in odor or color were detected in irradiated ostrich meat exposed to doses of 2, 4, and 6 KGY, even after 15 days of refrigerated storage.

It is important to note that while TVN levels experienced a significant increase on the 15th day of storage, irradiation appeared to have a decreasing effect on this index. However, this difference was not statistically significant. Overall, our study suggests that irradiation had no significant impact on the organoleptic characteristics, including odor and color, of the ostrich meat.

In the study conducted by Heydari and colleagues (2017), ostrich meat was treated with doses of 1.5, 3, and 5 KGY gamma irradiation. The results showed a significant reduction in the levels of nitrogen compounds and an increase in protein content compared to the control group. Additionally, the gamma irradiation treatment significantly reduced the levels of some essential minerals, including iron, in the ostrich meat. Furthermore, the application of gamma irradiation enhanced the safety and quality of the meat, making it more suitable for human consumption.(40)

In Khalida and colleagues' study in 2021, the effects of gamma irradiation and kale leaf powder (KLP) on the microbiological parameters (*Total Bacteria Count* and *Coliforms*) and quality parameters (Hunter color values L*, a*, and b*) of ostrich and chicken meat and meat products were assessed. The results indicated that irradiation, with or without different load compositions, minimized the *Total Bacteria Count* and substantially reduced *Coliformspp* contamination during storage in both types of meat and meat products. Moreover, the nutritional, qualitative, and sensory characteristics of the products were improved with gamma irradiation.(32)

In conclusion, the use of irradiation as a food preservation method, particularly for meat products like ostrich meat, is highly recommended. Irradiation can be regarded as a critical control point in the food supply chain, serving as an additional contamination control measure in the processing of raw animal-derived food products at slaughterhouses, meatpacking centers, and meat processing facilities. The application of irradiation for extending the shelf life of ostrich meat is encouraged, and it has the potential to be one of the most effective methods for ensuring the safety and quality of ostrich meat products.

It is advisable to explore the combined use of irradiation with other preservation techniques to further reduce microbial contamination and eliminate foodborne pathogens in ostrich carcasses. This holistic approach can enhance the overall safety of ostrich meat products. Therefore, it is essential to conduct further research in this area to address health concerns related to ostrich meat supply.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Safety and Quality of Beef Meat Sausages Produced in the Industrial Factory Using the HACCP System

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ABSTRACT

Introduction: Food safety and hygiene are important principles for food hygiene officials and the majority of large food industries around the world. The purpose of this experiment was to investigate the safety and quality of beef meat sausages produced in the local factories using the HACCP and non-HACCP systems.

Methods: One hundred and twenty samples of beef meat sausages from the non-HACCP and HACCP local meat product markets were examined for three months in terms of microbial (total viable count, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., coliforms, and mold/yeast) and chemical (total volatile basic nitrogen and pH) properties based on the HACCP standard of meat products.

Results: The levels of microbial population and chemical properties of raw materials and beef meat sausages in the HACCP factory samples were significantly lower than those of non-HACCP factory samples ($P < 0.05$). Moreover, 100% of the examined spices in HACCP factory were found to have microbial populations below the critical limit of plants, while 100% of the examined spices in non-HACCP factory was contaminated.

Conclusion: The results of the present study indicated that the HACCP principle effectively controls the microbial hazards and chemical property of prepared beef meat sausages.

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Introduction

Food safety and hygiene are important principles for food hygiene officials and the majority of large food industries around the world (1, 2). A weak food safety system leads to the spread and transmission of food-borne diseases with high morbidity and mortality rates (3, 4). Food products may suffer secondary microbial contamination during manufacturing, transportation, processing, and packaging (5). The World Health Organization (WHO) considers diseases caused by food contamination as one of the most important public health problems in the contemporary world (6). The consumed food may be completely in harmony with human physical needs and have all the conditions of adequate nutrition, but in terms of contamination and/or the presence of harmful microbial and chemical hazards, it seriously threatens human health (7, 8). The consequences of spoilage and contamination often occur due to the preparation and processing conditions of food products that can have adverse effects on human health either in the short term or in the case of continued consumption (9). According to the estimate made by the Center for Disease Control and Prevention in the United States of America, 75 million people suffer from food-borne diseases every year, more than 325,000 people are hospitalized, and 5,000 people die (5, 10). The annual cost of food-borne diseases, including direct medical costs and productivity loss in this country is approximately 5 to 6 billion dollars. Regarding *Salmonella* spp. infection, direct and indirect costs are estimated at approximately 1 billion dollars per year (6).

Management of food safety parameters, identification, evaluation, and control of risk factors in the food chains can lead to the prevention and reduction of microbial and chemical hazards, improve the quality and safety of food products, and ultimately provide safer food to consumers (2, 11). Various studies have shown that the microbial and chemical properties of different perishable foods can be improved by using food safety programs (7, 12-14). Moreover, the growing trend of the number of production units in the food industry and the

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changes in the technology and variety of products in the world have caused the owners of industries to make more efforts to establish quality systems (15-17). The Hazard Analysis Critical Control Points (HACCP) system was accepted by the Codex Commission in 1993 and was widely utilized in various food industries in countries, such as the United States of America and Japan (18, 19). In recent years, HACCP has been used as an effective control system at the global level (2). Therefore, the purpose of this experiment was to investigate the safety and quality of beef meat sausages produced in the local factories using the HACCP and non-HACCP systems.

Materials and Methods

Sampling

Cooked beef sausages were mainly prepared by defatted beef meat, ice water, wheat flour, starch, sodium polyphosphate, spices, ascorbic acid, sunflower oil, powdered milk, salt, guar gum, sodium nitrite, and wheat gluten (20). All the ingredients were mixed in the cutter machine and stuffed in polyamide casings. 120 samples of beef meat sausages from the non-HACCP and HACCP local meat product markets were examined for three months in terms of pathogenic and spoilage microbial agents based on the HACCP standard of meat products (12, 21). Sampling of beef meat sausages was conducted before and after sausage processing and packing. All obtained sausage samples were wrapped in aseptic bags, put in a piece of ice, and immediately transferred into the laboratory for microbiological and chemical analysis between 45 min and 1 h.

Microbial Analysis

For microbial analysis, an amount of 25 g beef meat sausages were homogenized at high speed for 3 min in a sterile bag mixer through the stomacher (BagMixer, Interscience, France) with 225 ml of 0.1% sterile buffered peptone water (Merck, Germany). The culture medium was sterilized by autoclaving for 15 min at 121 ± 2 °C. The homogenates prepared by the stomacher were serially diluted with sterile 0.1% buffered peptone water, and 0.1 ml was cultured on the plate count agar (incubated at 37 ± 1 °C for 48 h), eosin methylene blue agar (incubated at 37 ± 1 °C for 24 h), Baird Parker agar (incubated at 37 ± 1 °C for 48 h), *Salmonella-Shigella* agar (incubated at 37 ± 1 °C for 24 h), violet red bile agar (incubated at 37 ± 1 °C for 24 h), and sabouraud dextrose agar (incubated at 25 ± 1 °C for 7 days) to enumerate total viable count (TVC), *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., coliforms, and mold/yeast in meat products, respectively (5). All corresponding culture media were obtained from Merck, Germany.

Chemical Analysis

The beef meat sausages (10 g) were homogenized in the stomacher for 10 min with 90 ml of distilled water to make a thick slurry, and then the pH was determined using a digital pH meter (Farazbin, Iran) (22). To determine the total volatile basic nitrogen (TVB-N) content of the samples, 20 g of the sample was mixed with 200 ml distilled water, stirred for 12 min at 3400 rpm, filtered, and alkalinized by incorporating 5 ml MgO solution (10 g/l). The volatile base components were extracted through steam distillation using a Kjeldahl distillation unit for 10 min and obtained with 10 ml boric acid (20 g/l), and a few drops of 0.1% methyl red and bromocresol green indicators. Following that, the sample was titrated with 0.1 mol/l HCl. The TVB-N content was expressed as mg N/100 g (14).

Statistical analysis

All analysis was conducted three times. Statistical analysis of the results was done using Tukey's multiple comparison test through the SPSS program (version 21 for Windows, Chicago, IL, USA). The findings were exhibited as mean ± standard deviation. $P < 0.05$ was described as a statistically significant difference.

Results and Discussion

The Codex Alimentarius defines food hygiene as "all conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain". Prerequisite hygiene programs, including good hygiene practices (GHP) and HACCP are compulsory (2). Based on the results presented in Table 1, the levels of microbial population of raw materials in the HACCP factory samples were significantly lower than those of non-HACCP factory samples ($P < 0.05$). The higher microbial population of non-HACCP factory samples suggests the poor hygienic quality of the raw materials, inadequate handling, and storage practices (16, 23). Isolated coliforms in raw materials could be related to the existence of fecal contamination during the slaughtering process (24). All samples were negative for *Salmonella* spp. in the non-HACCP

and HACCP markets. Reduction of the potential existence of *E. coli* and coliforms in raw materials is crucial, since *E. coli* and coliforms can lead to serious public diseases (1). As previously reported, three important factors that affect the hygiene quality of raw materials are the situations under which animals are reared, slaughtered, and processed along with the intrinsic and extrinsic parameters of microbial growth in raw beef meat samples and spices (25). The results of TVC, *E. coli*, *S. aureus*, *Salmonella* spp., coliforms, mold/yeast, pH, and TVB-N of beef meat sausages prepared in the non-HACCP and HACCP markets are presented in Table 2. The levels of microbial and chemical properties of beef meat sausages prepared in the HACCP factory were significantly lower than those of prepared in the non-HACCP factory ($P < 0.05$). Based on our findings, the TVC, *E. coli*, *S. aureus*, *Salmonella* spp., coliforms, mold/yeast, pH, and TVB-N of beef meat sausages prepared in both non-HACCP and HACCP markets were in the acceptable ranges of national standards (26). Poumeyrol, et al., (2010) reported that the hazard analysis effectively controlled by good hygiene practices for numerous bacterial hazards, particularly *Listeria monocytogenes*, *Salmonella* spp., and *S. aureus* (12). Hwang, et al., (2011) also found that the levels of aerobic plate count, total volatile basic nitrogen, and total coliforms in fish samples obtained from the HACCP factory were significantly lower than those of fish samples obtained from the two non-HACCP factories (14). Metaxopoulos, et al., (2003) indicated that the utilization of the HACCP system might be considered appropriate but more impacts are necessary for the control of the microbial and chemical safety of the incoming compounds and processing (27). Manios, et al., (2015) (28) reported that the high microbial population in meat products could be a consequence of raw materials with a high initial microbial counts, poor hygiene conditions during processing and packaging, along with high temperatures in the processing lines.

Table 1. Microbial population (log CFU/g) of raw materials from non-HACCP and HACCP markets.

	TVC	*E. coli*	*S. aureus*	*Salmonella* spp.	coliforms	mold/yeast
Non-HACCP market						
Additives	5.30 ± 0.13[a]	2.21 ± 0.02	3.81 ± 0.03[a]	< 1	3.89 ± 0.04[a]	3.89 ± 0.03[b]
Spices	5.49 ± 0.24[a]	< 1	< 1	< 1	< 1	5.12 ± 0.02[a]
Batters	5.83 ± 0.02[a]	< 1	4.20 ± 0.02[a]	< 1	3.87 ± 0.02[a]	2.18 ± 0.15[a]
HACCP market						
Additives	4.11 ± 0.02[b]	< 1	2.12 ± 0.23[b]	< 1	2.45 ± 0.08[b]	< 1
Spices	3.27 ± 0.28[b]	< 1	< 1	< 1	< 1	2.15 ± 0.02[c]
Batters	4.18 ± 0.12[b]	< 1	2.81 ± 0.14[b]	< 1	2.40 ± 0.03[b]	2.01 ± 0.08[c]

[a-b] Means with different lowercase letters in the same column are significantly different between raw materials of non-HACCP and HACCP markets ($P < 0.05$). Data are shown as mean ± standard deviation.

Table 2. Microbial and chemical properties of beef sausages prepared in the non-HACCP and HACCP markets.

	non-HACCP market	HACCP market
TVC (log CFU/g)	3.96 ± 0.04[a]	2.17 ± 0.25[b]
***E. coli* (log CFU/g)**	< 1	< 1
***S. aureus* (log CFU/g)**	< 1	< 1
***Salmonella* spp. (log CFU/g)**	< 1	< 1
Coliforms (log CFU/g)	< 1	< 1
Mold/yeast (log CFU/g)	< 1	< 1
TVB-N (mg N/100 g)	14.56 ± 0.07[a]	7.34 ± 0.02[b]
pH	6.29 ± 0.07[a]	6.20 ± 0.14[a]

[a-b] Means with different lowercase letters in the same raw are significantly different between beef sausages of non-HACCP and HACCP markets ($P < 0.05$). Data are shown as mean ± standard deviation.

Previous studies reported that one of the possible sources of food product contamination is spices, which consist of very high levels of microorganisms, particularly spore-forming *Bacillus* spp. and more frequently, *Clostridium* spp., that both of them enhanced TVC of sausage samples produced in non-HACCP factory (2, 29). As a small amount of spices was utilized in beef meat sausages, the incorporated spices in the current experiment did not overall contribute greatly to spoilage microorganisms of the product, however, it is possible consisted of heat-resistant pathogenic bacteria (2, 30). Moreover, raw beef meat samples should be handled properly to prevent any potential microbial contamination of final meat products along with

the areas in which they are processed (31). The microbial population of raw materials is likely related to the handling of samples during defrosting, deboning, and transporting to the next processing stages, cross-contamination during processing, and lack of high hygiene conditions of working staff and equipment (32). Another potential way for higher microbial contamination of beef meat sausages in non-HACCP factories could be the degree of contamination of the personnel and the surfaces in the processing plants constitutes, which is considered an important risk factor and should be controlled (21, 32). Our findings showed that 100% of the examined spices in the HACCP factory were found to have microbial populations below the critical limit of plants. Moreover, 100% of the examined spices in the non-HACCP factory were contaminated, which could be owing to the fact that they were not appropriately prepared and sterilized.

Conclusion

The results of the present study indicated that the HACCP principle effectively controls the microbial hazards and chemical property of prepared beef meat sausages. Moreover, the main enhancement must be regarding the standardization of the raw materials used, processing of the meat products, and training of the working staff. The present study has been conducted on a small scale without consideration of all the processing steps of beef-cooked sausages. Therefore, further experiments should be conducted for microbial analysis of meat cut at all operational steps, including the slaughterhouse, processing line, and retail outlets. More research is also required on the HACCP principle of a wider range of beef meat products.

Competing Interest

The author declares no conflict of interest.

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Effect of Orange Peel Essential Oil as a Natural Preservative on Characteristics of Turkey Meat Stored in Refrigerator

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ABSTRACT

Introduction: The tendency to consume turkey meat and meat products is increasing due to its high nutritional value. But due to its perishability, many researchers are looking for new solutions to increase its storage time and maintain its quality. Thus the purpose of this study was to evaluate the chemical composition of orange peel essential oil (OPEO) and its effect on the microbial, physicochemical and sensory properties of turkey meat during 12 days of storage at refrigerator temperature.

Methods: The chemical composition of OPEO was identified using GC/MS device. Three groups of turkey meat samples (control, 0.5 and 1%) of OPEO were packed and kept in the refrigerator and at regular intervals (days 0, 3, 6, 9, 12) for microbial tests (total count of aerobic, Pcychrotrophic, Enterobacteriaceae, *Pseudomonas aeruginosa*, lactic acid, MIC and MBC), chemical (pH, TV-N, TBARS) and sensory (taste, aroma, appearance, texture and overall acceptance) were evaluated.

Results: The results of GC/MS showed the presence of effective compounds with antimicrobial and antioxidant activity, especially D-limonene (71.47%). The results of microbial tests showed that treatments of turkey meat containing 1% OPEO had a significant effect ($P<0.05$) on the reduction of the bacteria population compared to the treatment of 0.5% OPEO and control samples. The MIC for *Listeria monocytogenes* and *Pseudomonas aeruginosa* was determined as 4 mg/ml and MBC was determined as 8 and 4 mg/ml, respectively. Lower values of pH, TV-N and TBARS, the highest sensory scores in terms of taste, aroma, appearance, texture and general acceptability were obtained in turkey meat treatments containing orange peel essence compared to the control group.

Conclusions: It can be said that due to its antimicrobial properties OPEO can be used as a natural preservative to increase the shelf life and sensory improvement of turkey meat samples during storage at refrigerator temperature.

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Introduction

Due to the importance of food safety, efforts have been made to improve it in all governments and pay more attention to the healthiness of food with the gradual increase in the population and lifestyle changes (1). Providing needed food, especially animal protein, is one of the most important needs of today's society, and poultry farming plays a significant role in providing protein needs. One of the main factors to pay attention to in this industry is the high growth rate, low food conversion factor and high nutritional value compared to other animal meat. Also, due to the high need for protein sources, the change in the taste of human societies and its high quality, has led to the development of turkey breeding in the world and especially in Iran (2).

Turkey meat is type of white meat with good nutritional value. It has the lowest level of fat and cholesterol compared to beef and sheep meat and also contains minerals such as iron, zinc, copper, potassium, magnesium, phosphorus, manganese, and is a good source of vitamins ascorbic acid, thiamine, riboflavin, pentatonic acid, It is B12, B6 and A. According to the FAO report, Iran ranks third in Asia in terms of turkey meat production. Meanwhile, turkey meat is a suitable environment for the growth of pathogenic microorganisms and is highly perishable due to

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the presence of moisture, protein and high pH. During the storage period, exposure to oxidative, microbial spoilage and adverse organoleptic changes is considered as a limitation in the production and trade of this product. Often, microbial spoilage of poultry meat is caused by Gram-negative bacteria, thermophilic bacteria, lactic acid bacteria, yeasts, and several types of Gram-positive bacteria. (3,4,5). Spoilage of raw meat during storage in the refrigerator occurs due to two reasons: microbial growth and oxidative spoilage. Spoilage of fresh poultry meat is an economic loss for producers of this product. Therefore, developing methods to increase shelf life, safety and quality is an important issue that the poultry meat production industry is facing (6). The presence of light, oxygen, chemical characteristics of meat, storage temperature and processing methods affect fat oxidation and this has been proven (7).

The common strategy adopted to prevent quality loss due to fat oxidation, which has led to a decrease in nutritional value and meat quality, is the use of antioxidant compounds (8). The negative effects of artificial antioxidants such as mutagenicity, poisoning and carcinogenicity have made the use of natural antioxidants suitable as alternatives (9). In recent years, a lot of attention has been paid to the waste of factories producing juice and concentrates (jams, tomatoes, apples and grapes), which contain natural antioxidants and their positive effect on human health and their antioxidant properties have been proven (10, 11).

Citrus fruits are one of the most important fruit products in the world, which can protect human health with a variety of phytochemicals, and are a good source of vitamin C, folic acid, potassium and pectin (12). In order to improve the management of these wastes and create added value, new processes are carried out to recover them through fertilizer, pectin, essential oil and antioxidant compounds, biodiesel, biogas and bioethanol (13, 14). Citrus peel is a perishable material with a very low shelf life due to its high moisture content (60-75%) (12). It also has more polyphenols and ascorbic acid than fruit pulp (15). The presence of bioactive compounds in orange peel has made it a suitable alternative to artificial antioxidants; there have been many reports on the antioxidant properties of orange peel (16, 17, 19, 18, 20, and 21). orange peel is rich in flavonoids, alkaloids, carotenoids, phenolic acids, limonoids, coumarins and polyethoxylated flavones, it is very valuable and rarely found in other plants, also the extracted essential oil of orange peel is used in pharmaceuticals, health and food industries (22). Also, there is interest in developing and using citrus waste as antioxidant compounds in meat products, in order to increase oxidative stability and maintain meat quality for longer shelf life as a way to maintain food safety according to consumer demand (23). Therefore, in this study, the chemical composition of essential oil extracted from orange peel is evaluated on the microbial, physicochemical sensory and properties of turkey meat stored at refrigerator temperature.

Materials and Methods

Preparation of Essential Oil and Its Analysis

Orange peel was collected from the waste of juice shops in Amol, Iran and after drying, it was ground. After drying and then grinding the orange peel, the essential oil was extracted by a Clevenger machine (Ambala, India) and by steam distillation for 3 hours. And the essential oil analysis was done by Gas Chromatography-Mass spectrometer (GC/M) (Biobase, China) (24). To prepare different concentrations, The OPEO was dissolved in distilled water containing Tween 80 (0.2%, w_{OPEO}) (25).

Preparation of Turkey Meat and Studied Treatments

Turkey meat was purchased from supply centers in Amol, Iran. The meat was transported to the laboratory in ice cubes and prepared. Fillets weighing 10 g were manually prepared for the treatments. The samples were packed in zipped bags and stored in a refrigerator at 4°C. Turkey meat in 3 groups including, the first treatment (control group) is immersion of meat in sterile distilled water for 30 minutes, the second treatment is immersion of meat in a solution of 0.5% OPEO for 30 minutes and the third treatment is immersion of meat in solution 1% OPEO was applied for 30 minutes in 3 repetitions.

Microbial Tests

For this purpose, the microbial culture of the samples for the total count of aerobic bacteria, cold sores, Enterobacteriaceae, *Pseudomonas aeruginosa*, lactic acid bacteria, MIC and MBC were performed at 5 different times, i.e. day 0

(beginning of the study), 3, 6, 9 and 12 (end of the study).

Preparation of Dilution from Samples

To prepare serial dilutions and count bacteria, 10 g of the sample was weighed in sterile zippered bags containing 90 ml of sterile 0.1% peptone water and homogenized with the help of a Stomacher stirrer. 1 ml of the dilution prepared under sterile conditions was added to tubes containing 9 ml of sterile 0.1% peptone water and different dilutions were prepared in the same way.

Examining the Antibacterial Activity of MIC and MBC Essential Oil

To determine the MIC or the minimum inhibitory concentration, a sterile 96-well plate was used with the broth micro-dilution method. 100 µl of Muller Hinton Broth culture medium was poured into rows 1 to 10 of houses, and then 100 µl of essential oil was added to the first house of each row. Row 10 contains 100 µl of culture medium without essential oil, 100 µl of microbial suspension was added and kept in an incubator at 25 °C for 24 hours. In order to determine the MBC or the minimum lethal concentration, 100 µl of the prepared dilutions were cultured on Mueller Hinton agar medium and incubated in an incubator with a temperature of 37 ˚C for 24 hours (25).

Cultivation and Enumeration of Bacteria

For enumeration of aerobic and Pcychrotrophic bacteria The amount of 100 µl of dilution prepared from each sample was cultured on plates containing Plate count Agar (PCA; Merck, Germany) medium and kept in incubator at 37 °C for 48-72 h and 7 °C for 10 days respectively (26). In order to enumeration of Enterobacteriaceae, 1 ml from different dilutions prepared from each sample was transferred to empty plates, then 10 to 15 ml of VRBGA (Violet Red Bile Agar) culture medium, which has a temperature of approximately 45 ˚C, was added to the plate, the sample was mixed with the culture medium and after the medium cooled down culture, another layer of the same medium was added to the plate in the amount of 4 to 5 ml. After completely closing the environment, it was kept in incubator at 37 °C for 18-24 hours (6).

Plates containing Pseudomonas base agar culture medium were used for *Pseudomonas aeruginosa* and stored in 20 °C for 2 days (6). Also, MRS agar medium was used to count the lactic acid of bacteria and it was stored at 25 ˚C for 5 days. The results were reported as the log CFU/g (6).

pH and Total Volatile Basic Nitrogen (TV-N)

5 g of the sample was homogenized with 45 ml of distilled water for 1 minute. The reading was done using a pH meter (Janco, Taiwan) (27). Then the amount of 10 g of sample along with 2 g of magnesium oxide as a catalyst was done by adding 300 ml of distilled water inside the Kjldahl flask. An Erlenmeyer flask containing 25 ml of 2% boric acid and methyl red and methylene blue reagents was placed at the end of the device, and boiling of the contents of the kjldahl flask and distillation of the emitted gases, which are nitrogen bases reagents, were performed. Distilled solution with hydrochloric acid 0.01 molar per titer, and volatile nitrogen substances were calculated in terms of mg of nitrogen per 100 g of sample (28).

Thiobarbituric Acid Reactive Substances (TBARS)

5 g of sample was homogenized with 15 ml of deionized water in 50 ml tubes for 15 seconds, 1 ml of the solution was transferred to another tube and 2 ml of acetic acid was added to it. Then the mixture was vortexed and kept for 15 minutes in a bain-marie at 90°C. The sample was vortexes for 10 minutes after cooling, then centrifuged at 3000 rpm for 15 minutes at 5°C. The optical absorbance of the upper layer was read at a wavelength of 531 nm (29).

Evaluation of Organoleptic Characteristics (color and appearance, smell, taste and texture)

In order to check the sensory and organoleptic characteristics of the sample, a panel of 5 people, whose members were educated people present in the laboratory, was used, and for evaluation, a three-point hedonic scoring system (score 1 is very bad and score 3 is very good) was performed (30).

Statistical Analysis

Statistical analysis of the obtained data was done with spss software. First, the normality of the data was checked using the Kolmogorav-Smirnov test, and then the homogeneity of the variance of the data was performed using the Leven test. Repeated measure (ANOVA) test was used to compare the average number of bacteria in the study period between the groups.

Results

Chemical composition of orange peel essential oil

The results of the analysis of chemical compounds identified in the OPEO sample are presented in Table 1. Quantitative and qualitative results of the analysis of the chemical composition of OPEO prepared by gas chromatography-mass spectrometry (GC/MS) led to the identification of 10 chemical compounds with a total of 97.88%. The results showed that D-limonene (71.74%) is the main chemical compound identified in OPEO. The main compounds identified in OPEO are linalool (7.76%), valencen (4.23%), β- pinene (4.02%), α-pinene (3.86%), acetamide (4.23%), Octanal (2.02%) and other compounds such as phenol, β-cadinene, 2- and 6-octadiene were (0.98, 0.88, 0.56%), respective.

Table 1. Analysis results of the studied orange peel essential oil using GC/MS method

Relative percentage of compounds	Compounds	number
0.98	phenol	**1**
7.76	linalool	**2**
2.02	Octanal	**3**
4.02	β-pinene	**4**
71.47	D-limonene	**5**
0.56	2,6-octadiene	**6**
3.86	α-Pinene	**7**
2.10	Acetamide	**8**
4.23	Valencen	**9**
0.88	β-cadinene	**10**
97.88%	-	**Total**

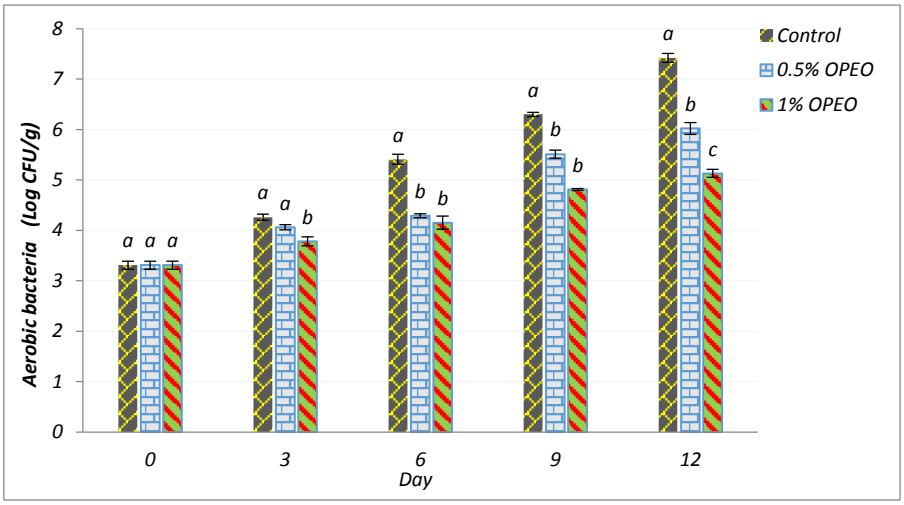

Figure 1. Results of the total count of aerobic bacteria in different treatments during storage (Mean ± SD)

Examining the Results of Microbial Tests

The results of total aerobic bacteria changes during storage are shown in Figure 1. The initial count of total bacteria in the present study increased significantly for the treatments over time ($P<0.05$). On the 0 days of the study, the bacterial population of the control group was 3.31 log CFU/g, which reached 7.42 log CFU/g (beyond the acceptable limit) at the end of the 12th day of storage. Total counts of aerobic bacteria in treated and control samples on 0 days were not significantly different from each other ($P>0.05$). The amount of bacteria in the treatment containing 0.5% and 1% OPEO on the 12th day of storage respectively was 6.02 log CFU/g and 5.13 log CFU/g, which were acceptable.

The results of changes in Pcychrotrophic bacteria during storage are shown in Table 2 and Figure 2. The amount of hypothermia for all treatments

increased significantly over time, while this increase was more intense in the control treatment. In all samples treated with different concentrations of OPEO, the number of hypothermia was significantly (P<0.05) lower was from the control group. The lowest count on the 12th day was observed in the group treated with OPEO of 1% of the Pcychrotrophic bacteria population (5.01 log CFU/g).

Table 2. The results of total count of Pcychrotrophic bacteria in different treatments during storage (Mean ± SD).

Treatment	Day				
	0	3	6	9	12
Control	3.11±0.06[a]	4.36±0.12[a]	5.08±0.14[a]	5.87±0.08[a]	7.09±0.07[a]
0.5% OPEO	3.11±0.06[a]	4.02±0.07[b]	4.25±0.08[b]	4.93±0.09[b]	5.81±0.11[b]
1% OPEO	3.11±0.06[a]	3.69±0.11[c]	4.11±0.10[c]	4.57±0.09[c]	5.01±0.06[c]

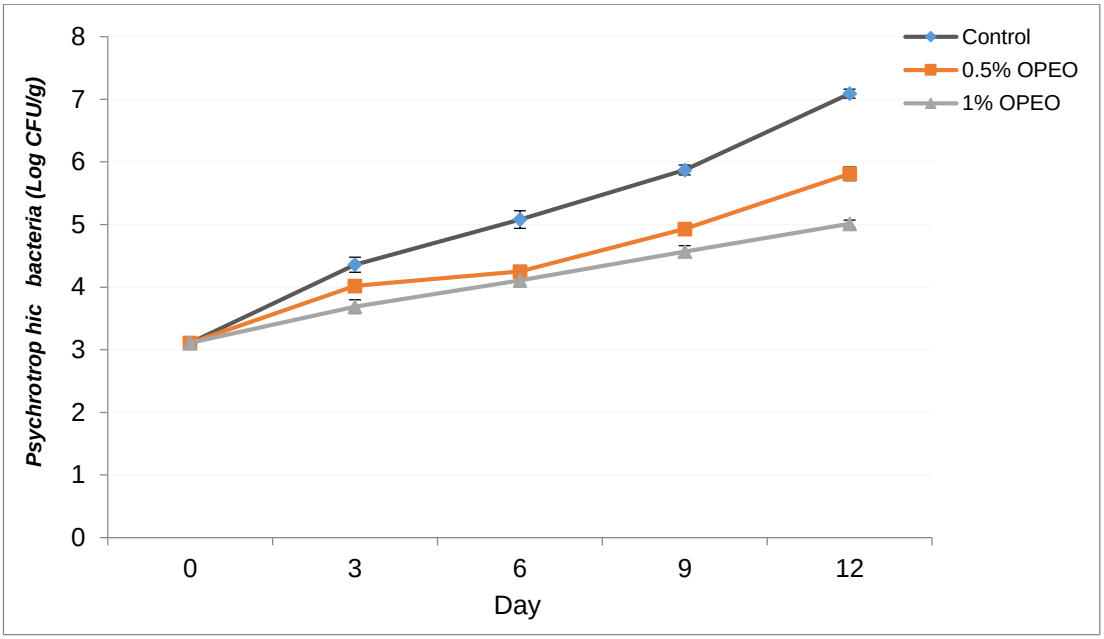

Figure 2. The results of total count of Psychrotrophic bacteria in different treatments during storage (Mean ± SD)

The results of changes in Enterobacteriaceae bacteria during storage are shown in Table 3. With the passage of storage time, the number of Enterobacteriaceae bacteria increased significantly for all treatments, reaching 6.39 log CFU/g in the control group on the last day of the study. In all samples treated with different concentrations of OPEO, the count of Enterobacteriaceae was significantly (P<0.05) lower than the control group. The results of the changes related to the counting of *Pseudomonas aeruginosa* species during storage are shown in Table 4. The number of *Pseudomonas aeruginosa* bacteria in the treated and control samples on 0 days did not differ significantly (P>0.05). In all study days, the population of *Pseudomonas aeruginosa* was significantly lower than the control group (P<0.05) in the groups treated with OPEO concentrations (0.5 and 1%). The results of the changes related to the counting of lactic acid bacteria are shown in Table 5. With time, the maintenance of the population of these bacteria increased over time for all treatments (it was the lowest on day 0 and the highest on day 12th) (P<0.05). So that this increase was more intense in the control sample and its value reached 5.60 log CFU/g. In the samples treated with 1% OPEO, it was significantly lower than the other two groups (P<0.05).

Table 3. The results of the total count of Enterobacteriaceae bacteria in different treatments during storage (Mean ± SD).

Treatment	Day				
	0	3	6	9	12
Control	2.16 ±0.05[a]	2.59±0.03[a]	3.99±0.04[a]	4.80±0.08[a]	6.39±0.087[a]
0.5% OPEO	2.16 ±0.05[a]	2.41±0.06[b]	3.03±0.06[b]	3.91±0.08[b]	5.12±0.13[b]
1% OPEO	2.16 ±0.05[a]	2.40±0.05[b]	2.78±0.07[c]	3.21±0.01[c]	4.15±0.12[c]

Table 4. The results of the total count of *Pseudomonas aeruginosa* bacteria in different treatments during storage (Mean ± SD).

Treatment	Day				
	0	3	6	9	12
Control	2.41 ±0.05[a]	4.02±0.02[a]	5.52±0.06[a]	6.42±0.05[a]	7.74±0.08[a]
0.5% OPEO	2.41 ±0.05[a]	3.68±0.07[b]	4.97±0.13[b]	5.87±0.09[b]	6.22±0.04[b]
1% OPEO	2.41 ±0.05[a]	3.11±0.12[c]	4.17±0.10[c]	4.71±0.03[c]	5.65±0.03[c]

Table 5. The results of total count of lactic acid producing bacteria in different treatments during storage (Mean ± SD).

Treatment	Day				
	0	3	6	9	12
Control	2.05±0.04[a]	2.55±0.02[a]	3.83±0.11[a]	4.53±0.02[a]	5.60±0.01[a]
0.5% OPEO	2.05±0.04[a]	2.37±0.04[b]	3.12±0.05[b]	3.46±0.05[b]	4.91±0.10[b]
1% OPEO	2.05±0.04[a]	2.35±0.05[b]	2.75±0.12[c]	3.10±0.04[c]	3.95±0.00[c]

The results related to the antimicrobial activity of OPEO by the agar whole method, the minimum growth inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on the investigated strains are given in Table 6. The results related to the minimum growth inhibitory concentration of *Listeria monocytogenes* and *Pseudomonas aeruginosa* bacteria were determined to be 4 mg/ml. Also, the results of OPEO MBC for two strains of *Listeria monocytogenes* and *Pseudomonas aeruginosa* were determined as 8 and 4 mg/ml.

Table 6. MIC and MBC results of orange peel essential oil

Bacteria	MIC(mg/ml)	MBC(mg/ml)
Listeria monocytogenes	4	8
Pseudomonas Aeruginosa	4	4

Examining the Results of Chemical Tests

Changes in the pH of turkey meat samples during storage are presented in Figure 3. The trend of pH in all groups was increasing, so this increase was more intense in the control sample and reached 7.03 on the 12th day. On 0 day, no significant difference was observed between the treatments ($P < 0.05$) in the samples treated with OPEO (1%), the pH value was significantly ($P < 0.05$) lower than the control group, which is On the 12th day, this amount reached 6.72.

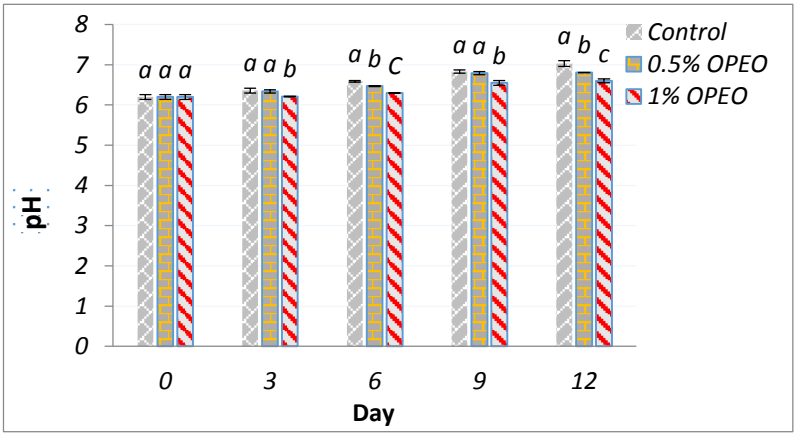

Figure 3. Average pH changes in different treatments (Mean ± SD)

The changes in the amount of total volatile (TV-N) of turkey meat samples during storage are reported in Figure 4 With the increase in storage time, the trend of the amount of total volatile nitrogen substances in all groups was increasing, so this increase in the control treatment compared to the other treatments was more and reached 60.8 mg/100g on the 12th day. In the samples treated with OPEO (1%), the amount of TV-N was significantly (P<0.05) lower than the control group, which reached 21.05 mg/100g on the 12th day.

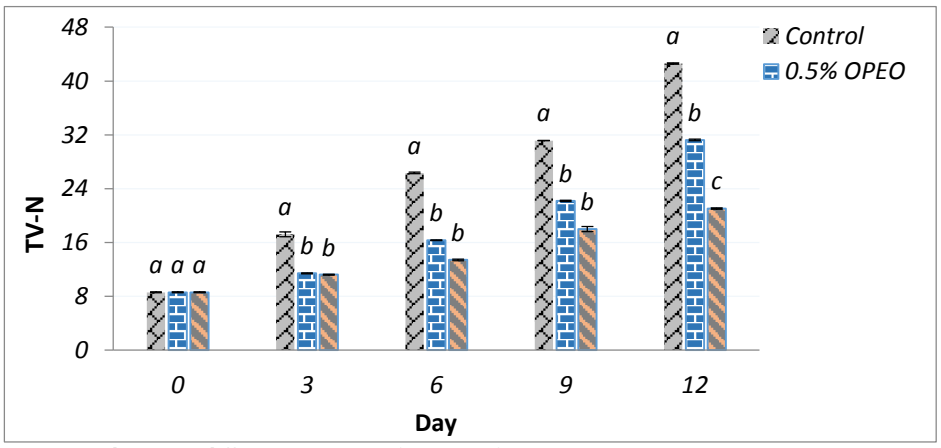

Figure 4. Average TV-N changes in different treatments (Mean ± SD)

Changes in Thiobarbituric acid of turkey meat samples during storage are shown in Table 7. With increasing storage time, the amount of Thiobarbituric acid in all samples increased (P<0.05). However, the increase in TBARS in OPEO samples (0.5% and 1%) was less than in the control group (P<0.05). On the 12th day of storage, the lowest TBARS values obtained in the 1% OPEO sample were observed, which was equal to 2.01.

Table 7. Average TBARS changes in different treatments (Mean ± SD)

Treatment	Day				
	0	3	6	9	12
Control	0.15±0.02^{a}	0.46±0.01^{a}	1.60±0.07^{a}	2.44±0.02^{a}	3.15±0.01^{a}
0.5% OPEO	0.15±0.02^{a}	0.27±0.01^{b}	1.02±0.04^{b}	1.93±0.00^{b}	2.66±0.04^{b}
1% OPEO	0.15±0.02^{a}	0.25±0.05^{b}	0.76±0.03^{c}	1.19±0.05^{c}	2.01±0.03^{c}

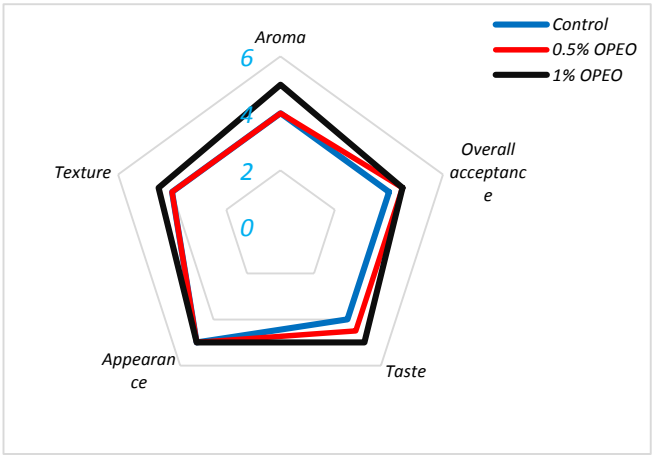

Figure 5. Sensory evaluation results of samples in different treatments

Sensory Evaluation

Changes in sensory characteristics (taste, aroma, appearance, texture and general acceptance) of turkey meat samples stored at refrigerator temperature are reported in Figure 5. During the maintenance period, the treatments obtained a higher and acceptable score in terms of sensory characteristics including taste, aroma, texture and overall acceptance ($P<0.05$) and no statistically significant difference was observed between the treatments in terms of appearance ($P>0.05$) According to the results shown, 1% OPEO treatment, compared to other treatments, it had more aroma, taste, appearance, texture and overall acceptance score during the storage period.

Discussion

According to the analysis of chemical constituents in OPEO in this study, D-limonene was the most abundant compound OPEO with 74.71%, which was lower than the values reported in previous researchers' studies (31, 32). Of course, the amounts of these compounds can be different due to different factors such as the type of essential oil extraction, plant variety, genetic factors, geographical location, climatic conditions, and soil. In line with the findings of this research, Khan et al. (2012) also reported D-limonene as the main compound in OPEO (33). There have been many reports regarding the antioxidant properties of citrus essential oil (5, 6, and 7). In meat and meat products, the highest allowed amount of aerobic bacteria count is 7 log cfu/g (34). In this study, the count of aerobic bacteria in the control sample did not exceed the maximum acceptable value until the 9th day, and the samples were treated with OPEO until the 12th day. The results of this study are in line with the results of Milani et al (2020) study, which stated that the use of edible gelatin-Hydroxypropyl β-cyclodextrin coating containing Nano-emulsion leads to a significant reduction in the number of aerobic bacteria in turkey meat (35). The highest amount of spoilage in the meat and meat products industry occurs through Pcychrotrophic bacteria, which are aerobic (36). In the present study, the number of Pcychrotrophic bacteria in the samples treated with the highest concentration of OPEO was reduced compared to the control sample. The reason for the low number of these bacteria is the presence of phenolic amounts in OPEO. The chemical structure and hydroxyl groups in them are factors that can play a role as an antimicrobial property in essential oils (37). Similar results were reported by Noshad et al. (1400) who showed that the number of Pcychrotrophic bacteria in the buffalo meat sample treated with fruit Cordia myxa mucilage-orange peel essence was lower than in the control group (38).

Enterobacteriaceae are present in large quantities in the meat industry and meat products and play their role as the main cause of spoilage and endangering people's health. In the present study, the population of Enterobacteriaceae in the samples treated with OPEO was lower than in the control sample. In a similar study by Fayaz far et al. (1400), it was reported that adding high concentrations of Shirazi thyme essential oil (0.1 and 0.2%) to fresh turkey sausages significantly reduces the number of Enterobacteriaceae during 17 days of storage. Also, as the concentration of essential oil increases, the population of bacteria decreases (39).

In the present study, the initial number of *Pseudomonas aeruginosa* bacteria in the groups treated with different concentrations of OPEO (0.5% and 1%) was lower than the control group, so their count on the twelfth day of storage in the treatment with 1% OPEO was 4.15 log cuf/g arrived. The results of this research were in line with the study of Fayaz Far et al. (1400), which showed that the population of *Pseudomonas bacteria* in concentrations (0.2 and 0.1%) of Shirazi thyme essential oil to fresh turkey sausages was lower than the control group (39).

Lactic acid bacteria are the main spoilage organisms in vacuum or low oxygen and their large number causes spoilage and discoloration in meat. In the present study, the population of lactic acid bacteria in samples containing OPEO decreased significantly compared to the control sample of turkey meat. The results of counting lactic acid bacteria were consistent with the results of Vasiliki et al. (2016) (40). In this study, the results of (MIC) of OPEO for *Listeria monocytogenes* and *Pseudomonas aeruginosa* were determined as 4 mg/ml. In the study of Oraii et al. (2018), the MIC level of the ethanolic extract of orange peel in vitro for *Pseudomonas aeruginosa bacteria* was 5% (41) and in the study of Noshad et al. (2019) for *Listeria bacteria*, it was less than 4 mg/ml. (38). In the present study, the results of MBC of OPEO for *Listeria*

monocytogenes and *Pseudomonas aeruginosa* were determined as 4 and 8 mg ml. Yun-Chen et al. (2008) stated that there are flavonoids, pectin, carotenoid and phenol in orange peel, which can have antimicrobial properties (42). In the present study, OPEO can have a significant antibacterial effect on both strains. In a similar study, Milani et al. (2019) reported the MBC of these two bacteria as 1.250 mg/ml and 0.625 mg/ml. In the present study, the increase in pH in the samples treated with OPEO was much lower than in the control sample, which could be due to the antioxidant activity of OPEO. Taheri et al. (2016) also conducted a study on the effect of acetic acid on turkey and stated that there was a slight increase in the pH value of the treated samples (6.21), but this increase in the control sample (7.03) quickly has been more (34). The reason for the increase in the pH value in the control sample is the increase in the number and activity of microorganisms, which can affect the proteins and the separation of amino compounds. Ali Beigi et al. (2012) studied the antioxidant effect of orange peel extract on the quality of carp fillets and reported that the pH increased with increasing storage time, and this trend was higher in the control treatment. During the beginning of storage, a decrease in pH occurs due to the breakdown of glycogen and the formation of inorganic acids (such as lactic acid) and leads to the inhibition of the growth of microorganisms (44).

The amount of volatile nitrogen (TV-N) is used to determine the quality of food of animal origin. Factors such as autolysis (self-digestion) of meat protein and the increase in the number of game compounds during the storage period cause a bad smell, and the role of this quality index is to help determine and evaluate the quality of the product because the increase in its amount decreases the duration. The maintenance and activity of spoilage bacteria and internal enzymes are related (45).

According to the results obtained in the present study, the amount of volatile nitrogen in the control group up to day 6, as well as in the OPEO treatment of 0.5% up to day 9, and in the OPEO treatment of 1% up to day 12, was lower than the standard limit by the country's veterinary organization (27 mg/100g). The results of this research were similar to the results of Taheri et al.'s study (2016) regarding the effect of acetic acid on reducing the amount of TV-N in the treatment of turkey meat fillets (43). The process of fat oxidation in meat, in which unsaturated fats are oxidized by free radicals, can play an important role in meat color (46).

The TBARS index is related to measuring the amount of malonaldehyde, which is a secondary product of the oxidation of unsaturated fatty acids. Based on the results obtained in the present study, regarding the antioxidant activity of OPEO, the treated samples showed lower amounts of TBARS during the storage period compared to the control sample. In Kang et al.'s (2006) study, the amount of TBARS in the sample treated with OPEO was lower compared to the control sample due to the prevention of the essential oil from fat oxidation (10). In 2008, Tiets et al. stated the amount of 3 mg malondialdehyde /kg of fat as spoilage in meat. The results of the present study were consistent with the findings of Milani et al. (2020) who stated that the use of nettle essential oil nanoemulsion leads to a significant reduction of TBARS values in turkey meat (35).

In this study, the results of evaluating the sensory scores of turkey meat samples treated with OPEO compared to the control sample were in more favorable conditions in terms of all factors, and this antioxidant effect of OPEO on preventing the growth of microorganisms, improving quality and increasing storage time it shows samples containing essential oil (47). In the study of Ali Beigi et al. (2012), investigating the effect of orange peel extract on the quality of carp fillets, adding orange peel extract (0.5%) led to an increase in sensory properties and shelf life and also reported that fat oxidation was delayed (44).In another similar study, Naseri et al. (2018) stated that adding Chovir essential oil, in addition to inhibiting the growth and proliferation of microorganisms, increases the shelf life and improves the sensory characteristics of turkey meat (48).

Conclusion

The result of this research showed that OPEO, having natural antimicrobial and antioxidant properties and suitable sensory properties, has the ability to be effective and usable in order to increase the shelf life and improve the sensory properties of turkey meat. Among all the samples of turkey meat examined during 12 days of storage in refrigerated conditions, it was found that the sample treated with a concentration of

1% has a favorable effect on the microbial and chemical characteristics in order to reduce the total count of aerobic bacteria, Pcychrotrophic bacteria, Enterobacteriaceae bacteria, and *Pseudomonas Aeruginosa* and lactic acid bacteria and MBC in comparison with MIC on *Listeria monocytogenes* and *Pseudomonas Aeruginosa* also, there was a decrease in pH, TV-N and TBARS indicators during the storage period. In addition, they had a good and acceptable score in terms of sensory characteristics useful for studying. Therefore, the attention and use of citrus peel essential oil as a preservative in the food industry can reduce waste and create added value.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Nanocomposite Films Based on Soy Protein Isolate-Montmorillonite Nanoclay Containing Emulsion and Nanoemulsion of Zataria Multiflora Essential Oil for Preserving Chilled Chicken Burgers

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ABSTRACT

Introduction: This study aimed to evaluate the effect of nanocomposite film based on soy protein isolate-montmorillonite nanoclay (SPI-MMT) containing *Zataria multiflora* essential oil emulsion (ZEO) and nanoemulsion (ZNE) on the quality of chilled chicken burgers.

Method: Nanoemulsion, nanocomposite film, and chicken burgers were prepared based on instructions. The hamburgers were divided into six different groups with four replicates. The experimental groups were Control, SPI-MMT, SPI-MMT+1% ZEO, SPI-MMT+2% ZEO, SPI-MMT+1% ZNE, and SPI-MMT+2% ZNE, which were analyzed for microbial, physicochemical, and sensory parameters during 16 days of storage at refrigerator (days include 0, 4, 8, 12, and 16).

Result: The treated groups, including SPI-MMT+1% ZEO, SPI-MMT+2% ZEO, SPI-MMT+1% ZNE, and SPI-MMT+2% ZNE, showed the lower mesophilic and psychrophilic bacteria, lactic acid bacteria (LAB) and *Enterobacteriaceae* count than the control and SPI-MMT groups during storage. The treatments also reduced the increasing rate of total volatile nitrogen, lipid oxidation, pH, and cooking loss during storage. The SPI-MMT+2% ZNE treatment was the best treatment to reduce the microbial population, retard physicochemical and sensory changes, and increase the shelf-life of chicken burgers.

Conclusion: Based on the results, the nanocomposite film based on soy protein isolate-montmorillonite nanoclay containing *Z. multiflora* essential oil emulsion and nanoemulsion can improve the microbiological and physicochemical quality and is recommended for the preservation of chicken burgers during chilled storage.

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Introduction

Chicken meat is among the most popular types with desirable nutritional properties and a good source of high-value protein, minerals, and vitamins. The cholesterol content of chicken meat is lower than red meats, which increases its nutritional value and is preferred to other meat types in terms of health benefits and nutritional properties (28). The increasing world population produces chicken meat products with different ingredients and sensory characteristics. Burgers are the most common meat products consumed worldwide (25).

Microbial growth and oxidation of fats and proteins reduce the storage life of meat products during storage. There are various methods to preserve meat products. Edible films are packaging made from renewable, biocompatible, and biodegradable materials such as polysaccharides, proteins, and lipids and are one of the primary ways to control microbial and physicochemical changes in foodstuffs (10, 33).

The protein films possess higher mechanical properties than those made from carbohydrates and lipids while improving the nutritional value of foods (9). Soy protein provides a coating with a uniform and flexible texture, highly resistant to the penetration of oxygen and fat (8). Soy protein isolate (SPI) powder is prepared from defatted soybean flakes, washed in alcohol or water to remove the sugars and fiber, and then dehydrated and powdered. The protein content

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of SPI is more than 90% based on dry weight (24). Coating fried meat products with SPI reduces oil absorption and prevents moisture loss (5).

Nanocomposites are essential in improving polymer films' mechanical, physicochemical, and thermal properties compared to pure polymers or conventional composites (17). Nanoclays, such as hectorite, saponite, bentonite, and montmorillonite, have a particular surface area and unique properties in combination with biopolymers. Montmorillonite is one of the nanoclays that has recently received particular attention (3, 35).

Many studies have been done to replace chemicals with natural compounds to eliminate or reduce synthetic additives in foods, among which essential oils are a new way to preserve food (2). Shirazi thyme (*Zataria multiflora*) is a medical plant from the *Lamiaceae* family that grows in Iran (19), and its essential oil has various antiseptic, anesthetic, anti-epileptic, antibacterial, and antioxidant properties (4, 30).

Essential oils face several challenges, including increasing their stability and controlling their release during storage. In this regard, nanotechnology-based methods such as nanoemulsions are proposed. Nanoemulsions are more stable than conventional emulsions and have good physicochemical properties, which can be used to extend the storage life of commercial foods. On the other hand, the transfer of active compounds through biological membranes of nanoemulsions is higher and enhances the bioavailability of compounds (23).

This study aimed to evaluate the effect of nanocomposite film based on soy protein isolate-montmorillonite nanoclay containing emulsion and nanoemulsion of *Z. multiflora* essential oil on the quality of chilled chicken burgers.

Materials and Method

Materials

The fresh chicken burger was prepared by Dorsa slaughterhouse in Markazi province, Iran. Thyme (*Z. multiflora*) essential oil was obtained from Barij Essence Company (Kashan, Iran). Soy protein isolate was purchased from Barg-e-Sabz Company (Tehran, Iran). Monomorillonite (sodium form) was bought from Pishgaman Nanomaterials Company (Mashhad, Iran). All chemicals were obtained from Merck (Darmstadt, Germany). Microbial culture media were purchased from Condalab (Madrid, Spain).

Preparation of Nanoemulsion

The ingredients of the aqueous coarse emulsion were Tween 80 (4.5% w/w) (as surfactant or emulsifier) and *Z. multiflora* essential oil (6% w/w). High-speed homogenization (IKA, model T25D, Ultra Turrax, Staufen, Germany) at 10,000rpm for 15 minutes was used to prepare nanoemulsion of *Z. multiflora* essential oil (6).

Particle Size Measurement

The nanoemulsion's particle size and polydispersity index were measured using dynamic light scattering (HORIBA Scientific, model SZ-100, USA) (18).

Preparation of Nanocomposite Film

First, 5g of soy protein isolate (SPI) powder was added to distilled water and became uniform. The pH was adjusted to 10.5 with sodium hydroxide. Next, montmorillonite powder (1, 3, 5, 7, and 9%) was mixed with 1.25g glycerol as a plasticizer and mixed by a magnetic stirrer for 1h. The film solution (10ml) was poured into plates and was dried at 40°C for ~ 4h. Films with different montmorillonite ratios were evaluated regarding tensile strength and elongation percentage, and the most desirable film regarding these two factors was the film containing 5% montmorillonite. Finally, emulsion and nanoemulsion of essential oil (1% and 2%) were added to the SPI solution containing 5% montmorillonite. The final solutions were poured into trays and dried at 40°C for 4h, and the films were separated from the trays (15).

Chicken Burger Preparation

The sample was produced based on the formulation of the chicken burger and Iran's national standards (21). The fresh minced chicken breast meat was mixed with onion, breadcrumbs (8%), mixed spices (1%), and liquid oil (5%) for 5 minutes. The samples were placed between two films and divided into six groups after cutting the burger pieces to 100g (1 × 8cm). The first and second groups were the control and soy protein isolate-montmorillonite films (SPI-MMT), respectively. The third group was wrapped in soy protein isolate-montmorillonite film containing an emulsion of *Z. multiflora* essential oil with a concentration of 1% (SPI-MMT+1% ZEO). The fourth group was

wrapped in soy protein isolate-montmorillonite film containing an emulsion of *Z. multiflora* essential oil with a concentration of 2% (SPI-MMT+2% ZEO). The fifth group was wrapped in soy protein isolate-montmorillonite film containing nanoemulsion of *Z. multiflora* essential oil with a concentration of 1% (SPI-MMT+1% ZNE). The sixth group was wrapped in soy protein isolate-montmorillonite film containing nanoemulsion of *Z. multiflora* essential oil with a concentration of 2% (SPI-MMT+2% ZNE). The samples were stored in a refrigerator (3±1°C) and tested for microbial, physicochemical, and sensory changes for 16 days.

Microbial Analysis

About 10g of the chicken burger was homogenized with 90ml of sterile normal saline. The pour-plate method was used for counting total mesophilic bacteria (TMB) and total psychrophilic bacteria (TSB) in plate count agar (PCA) after the preparation of serial dilutions. The mixture was incubated at 35°C for 72h and 7°C for ten days, respectively. The lactic acid bacteria (LAB) were enumerated in MRS agar and incubated at 35°C for 72h. Violet red bile dextrose agar (VRBA) was used after incubation at 35°C for 24h to count *Enterobacteriaceae*. The colonies were reported as log CFU/g chicken burgers (18).

Physicochemical Analysis

The pH (7), total volatile basic nitrogen (TVN), thiobarbituric acid reactive substances (TBARS) (10), and cooking loss (29) were determined based on the procedures previously characterized.

Sensory Evaluation

The sensory properties of the chicken burgers were determined by 15 trained panelists using a 7-point hedonic scale. In this method, a score of 7 shows "excellent," and a score of 1 indicates "very poor" (34).

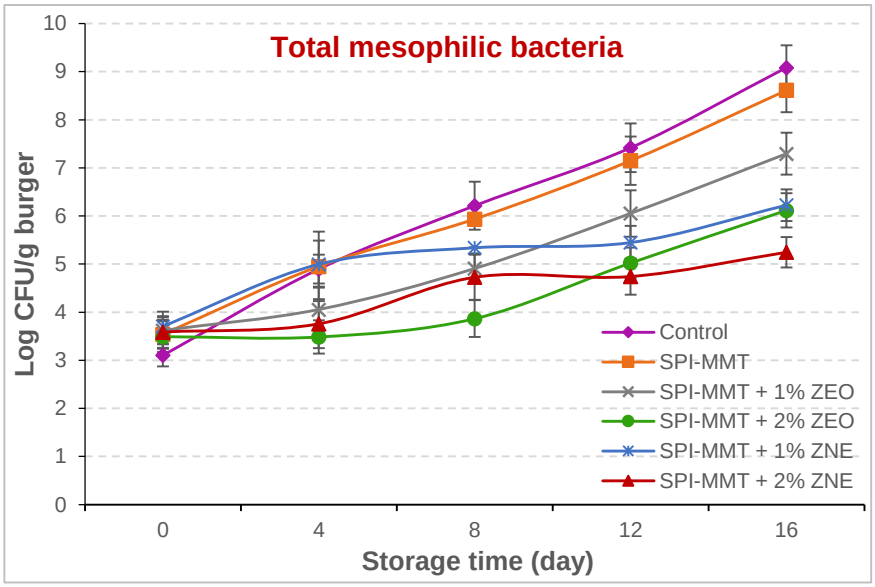
Statistical Analysis

The data of measured parameters were analyzed using analysis of variance followed by the Duncan post-test in SPSS software version 20. The statistical significance of all the variables was determined at the 5% probability level (p<0.05).

Results

Particle Size of ZNE

The mean particle size and polydispersity index of ZNE were 82.7 and 0.385nm, respectively.



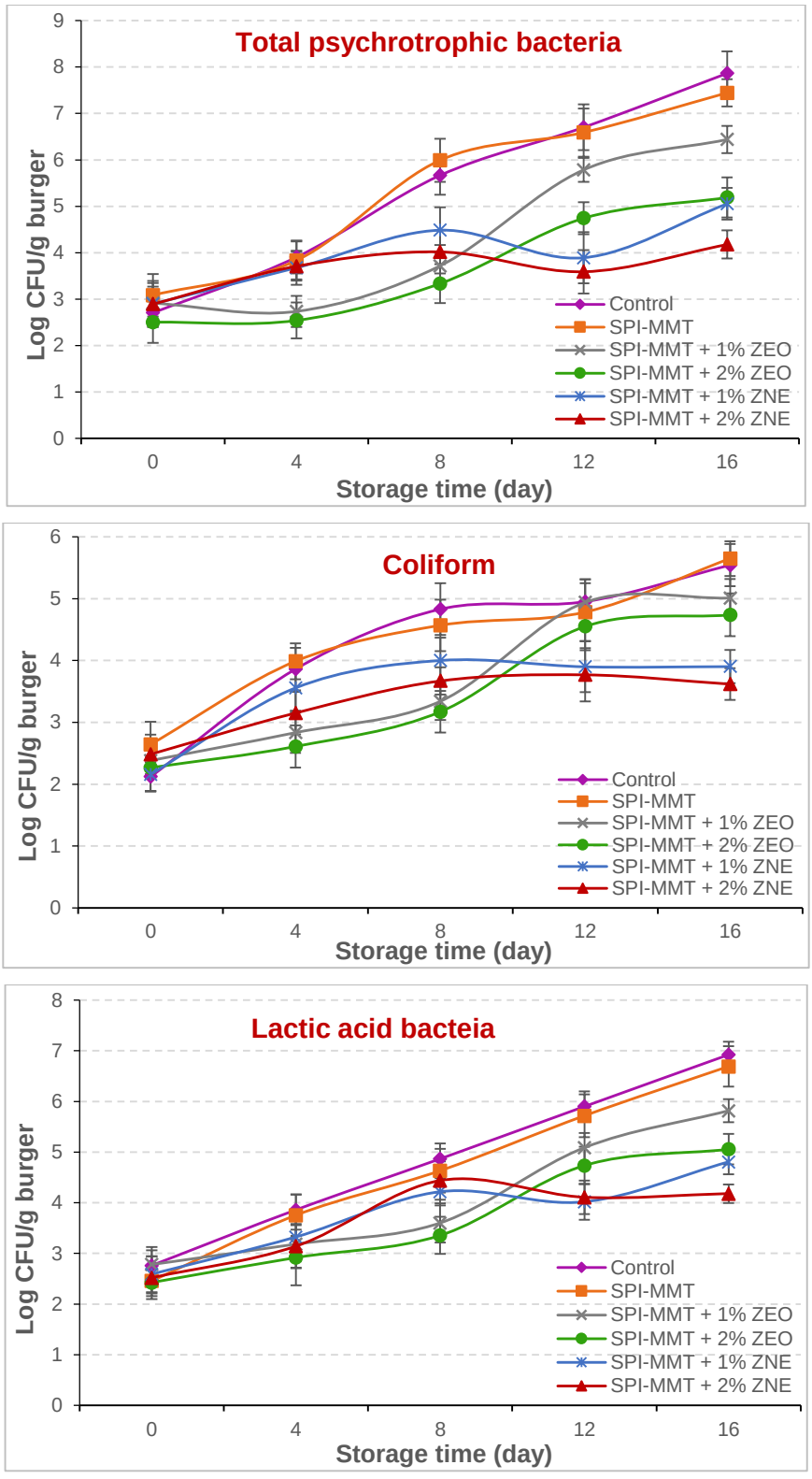
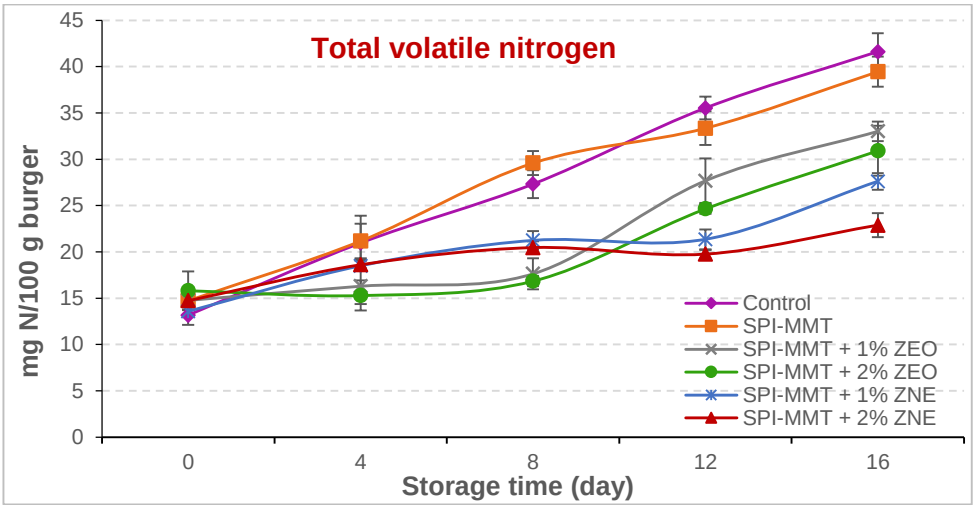
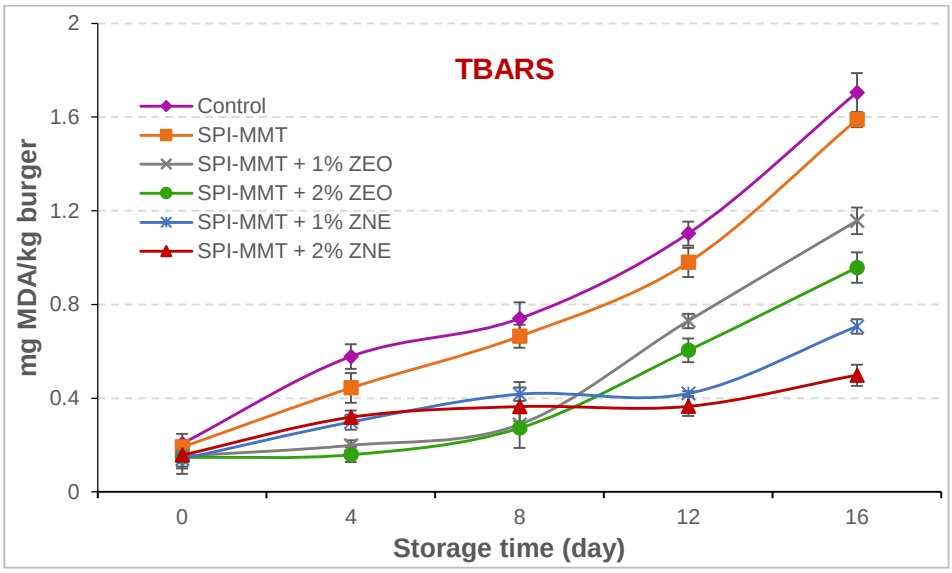


Figure 1. Microbial parameters of chicken burgers treated with SPI-MMT, ZEO, and ZNE

Microbiological Analyses

The results of microbial analysis for chicken burger samples are shown in Figure 1. At the beginning of the test (day 0), no significant difference ($p>0.05$) was observed between the TMB, TSB, LAB, and Enterobacteriaceae counts among the different groups. During storage, TMB and TSB increased in all groups (Figure 1a, b). On day 16, TMB and TSB were significantly higher in the control and SPI-MMT groups than in the treated groups ($p<0.05$). Samples containing free essential oil (SPI-MMT+ZEO) reduced the microbial population until day 8. Then, the function of samples containing nanoemulsion essential oil (SPI-MMT+ZNE) was better due to the gradual and controlled release of essential oil during storage in these groups (10). SPI-MMT+2% ZNE was the best group for controlling TMB and TSB in burgers. According to Iran's national standard, the limit of TMB in burgers is 6 log CFU/g burgers, which was higher than the standard limit on day 8in the control samples. In SPI-MMT+2% ZEO, SPI-MMT+1% ZNE, and SPI-MMT+2% ZNE groups, TMB did not exceed the standard limit until the end of the experiments (16 days).





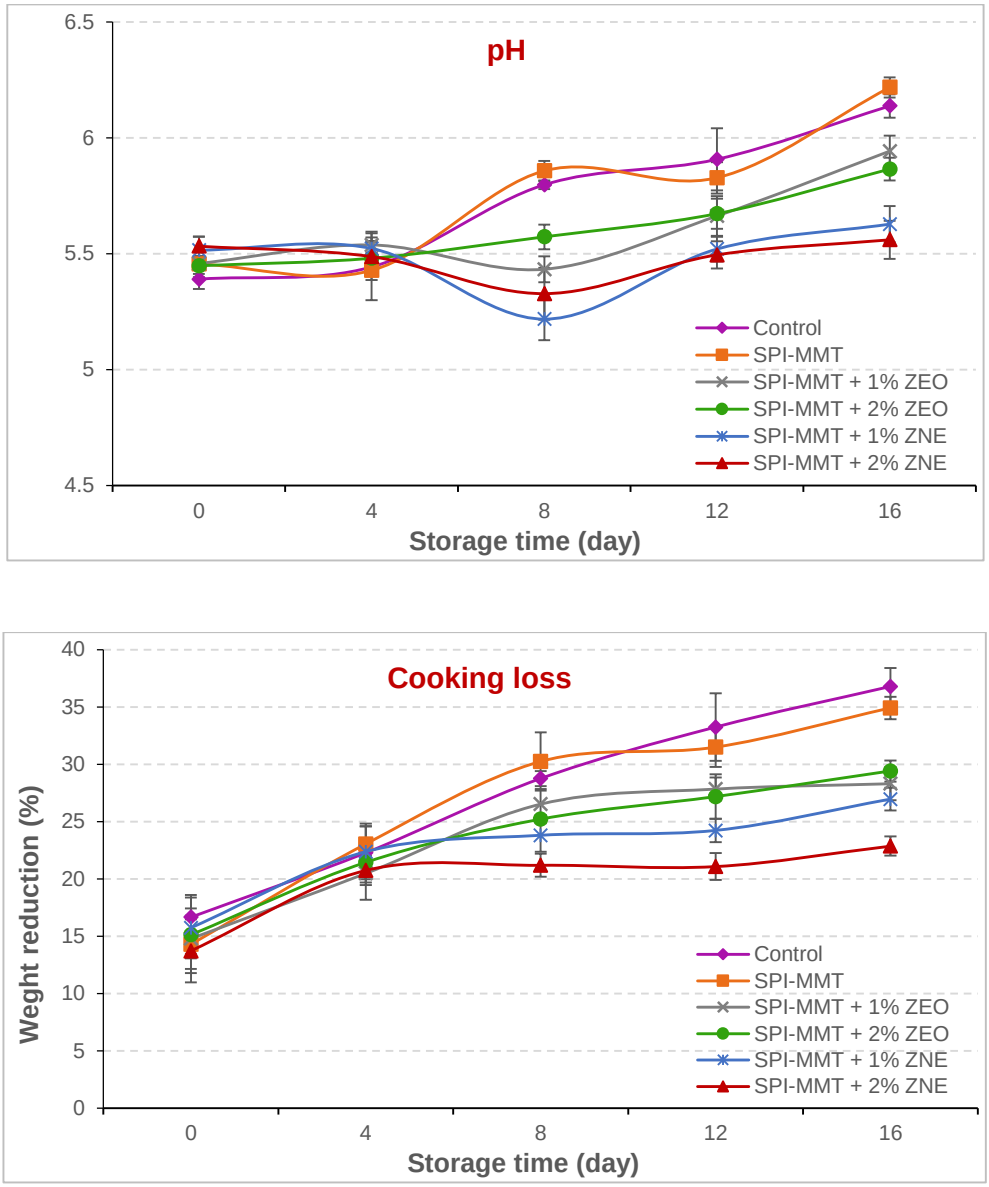


Figure 2. Physicochemical parameters of chicken burgers treated with treated with SPI-MMT, ZEO, and ZNE.

Physicochemical Analyses

A similar pattern was shown for the results of chemical parameters. TVN is a spoilage indicator that contains primary, second, and third amines (31). At day 0, TVN content and TBARS were not significantly different ($p>0.05$) in different groups (Figure 2a, b). The initial content of TVN in the studied groups ranged from 13.16 to 14.73mg N/100g burger, indicating the high quality of the burger samples. TVN content increased significantly during the storage period ($p<0.05$), but this increase was slower in the treated samples than in the control sample due to inhibition of microbial activity (28, 10, 18). SPI-MMT+2% ZNE showed the lowest TVN during storage. On day 8, the TVN content exceeded the standard limit in the control and SPI-MMT groups (25mg N/100g burger). In SPI-MMT+2% ZEO, SPI-MMT+1% ZNE, and SPI-MMT+2% ZNE groups, TVN content did not exceed the standard limit during 16 days.

The TBARS showed the lipid oxidation by-products, especially aldehydes (18). Initial TBARS levels were 0.20-0.15mg MDA/kg in all

groups and gradually increased during storage. At day 16, TBARS in SPI-MMT+2% ZNE was significantly lower than the other groups ($p<0.05$).

There was a more significant reduction in TVB-N and TBARS levels in samples containing free essential oil (SPI-MMT+ZEO) than in samples containing essential oil nanoemulsion. From the next day, the TVB-N and TBARS content was lower in samples containing essential oil nanoemulsion (SPI-MMT+ZNE) due to the controlled release of essential oil (10).

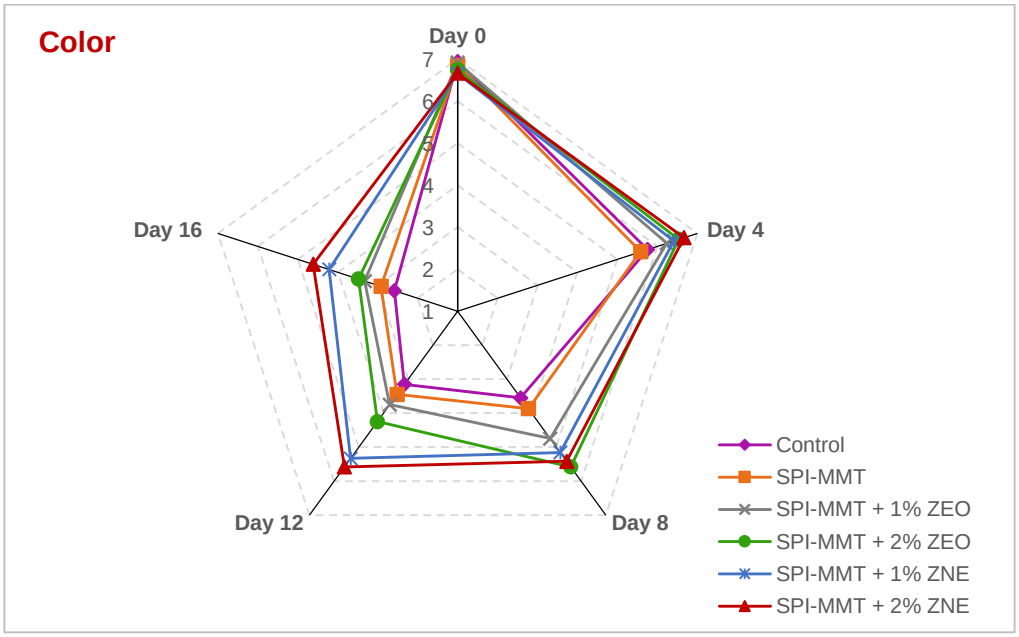
PH

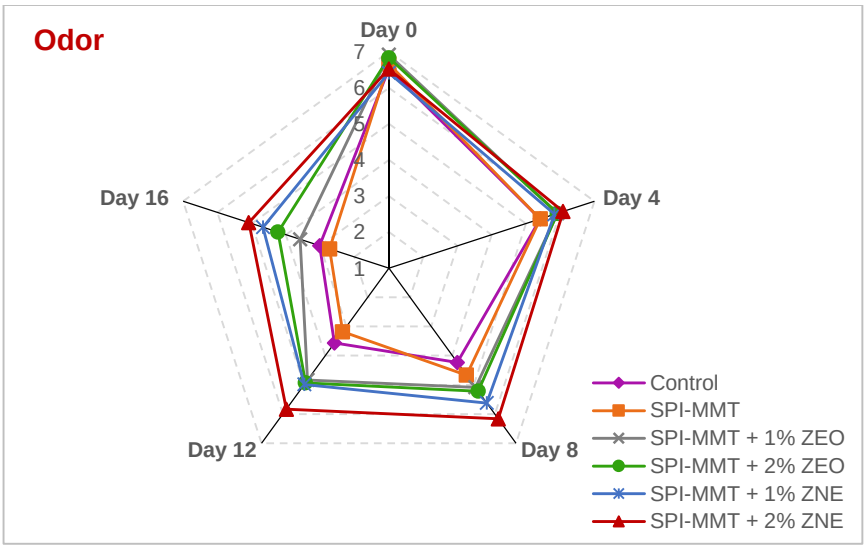
No significant difference was found in pH value among the experimental groups at days 0 and 4 ($p>0.05$) (Figure 2c). Then, the pH value was significantly higher in the control and SPI-MMT groups compared to the other groups ($p<0.05$). In all nanoemulsion groups (SPI-MMT+1% ZNE and SPI-MMT+2% ZNE), changes in pH were not significantly different at the end of the experiments. In contrast, in emulsion groups, the pH changes were significant ($p<0.05$). MMT+1% ZNE and SPI-MMT+2% ZNE were the best treatments to control pH changes in burger samples.

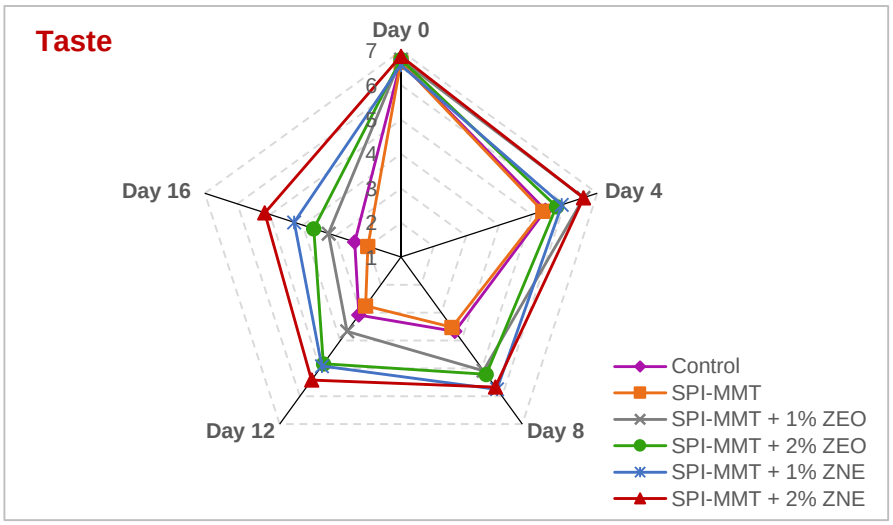
Cooking Loss

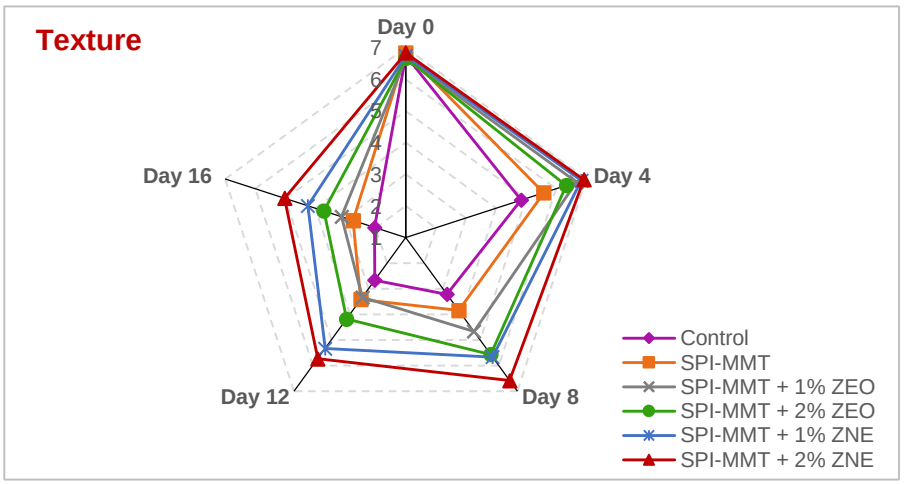
Juiciness and cooking loss are negatively correlated, implying that a high cooking loss results in low juiciness. Juiciness variation is partly explained by cooking loss, but it also influences the appearance of meat. A high cooking loss gives an expectation of a less optimal eating quality. There is also significant economic importance to cooking losses in the catering industry (11). The cooking loss is a combination of liquid and soluble matters lost from the meat during cooking and is calculated as the difference in weight between the uncooked and cooked burger divided by the weight of the uncooked burger (29).

Until the fourth day of storage, the cooking loss in different groups was not remarkably different ($p>0.05$), but there was a considerable increase during the storage ($p<0.05$), and this increase was slower in the treatment groups (Figure 2d). SPI-MMT+2% ZNE was the most effective treatment to reduce the cooking loss in burgers over 16 days of storage.




Odor
Day 0
Day 4
Day 8
Day 12
Day 16
7
6
5
4
3
2
1
Control
SPI-MMT
SPI-MMT + 1% ZEO
SPI-MMT + 2% ZEO
SPI-MMT + 1% ZNE
SPI-MMT + 2% ZNE


Taste
Day 0
Day 4
Day 8
Day 12
Day 16
7
6
5
4
3
2
1
Control
SPI-MMT
SPI-MMT + 1% ZEO
SPI-MMT + 2% ZEO
SPI-MMT + 1% ZNE
SPI-MMT + 2% ZNE


Texture
Day 0
Day 4
Day 8
Day 12
Day 16
7
6
5
4
3
2
1
Control
SPI-MMT
SPI-MMT + 1% ZEO
SPI-MMT + 2% ZEO
SPI-MMT + 1% ZNE
SPI-MMT + 2% ZNE

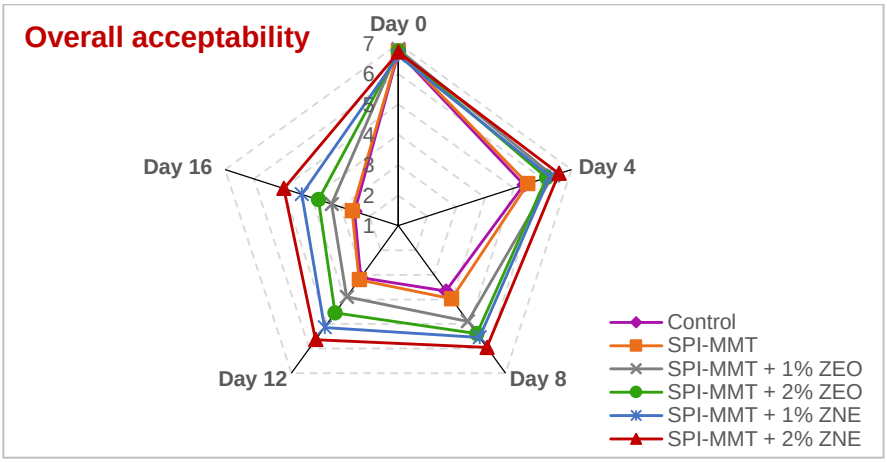


Figure 3. Sensory properties of chicken burgers treated with treated with SPI-MMT, ZEO, and ZNE.

Sensory Evaluation

The sensory analysis for chicken burgers is shown in Figure 3. the sensory parameters were highly desirable in all samples at the beginning of storage (scores≥6.5). The control sample was acceptable in terms of color, texture, taste, and overall acceptability until day 4, but it was acceptable from the point of view of odor until day 8. SPI-MMT+2% ZNE and SPI-MMT+1% ZNE groups were acceptable until day 16, but SPI-MMT+2% ZEO and SPI-MMT+1% ZEO were acceptable until days 12 and 8, respectively.

Discussion

This study showed that TMB and TSB increased in all experimental groups. On day 16, TMB and TSB were significantly higher in the control and SPI-MMT groups than in the other treated groups ($p<0.05$). Samples containing free essential oil (SPI-MMT+ZEO) reduced the microbial population until day 8. However, the function of samples containing nanoemulsion essential oil (SPI-MMT+ZNE) was better due to the gradual and controlled release of essential oil during storage in these groups. Dini et al. (10) showed that composite chitosan films containing nanoemulsion are more effective in reducing mesophilic and psychrophilic bacteria in beef loins. Hasani-Javanmardi et al. (18) showed that nanoemulsions reduced TMB, TSB, LAB, and Enterobacteriaceae in the lamb loins. Hassanzadeh et al. (20) reported similar results concerning chitosan-coated chicken breast containing grape seed extract. The results agree with other researchers who reported decreased mesophilic bacterial counts in coated beef with caseinate-whey protein (22).

During 16 days of storage, LAB counts increased in all groups, while the rate of increase was higher in the control group than in the treatment group (Figure 1c). SPI-MMT+2% ZNE group was the best sample for LAB control in burger samples. These results are consistent with those of Sarmast et al. (31), in which coated trout fillets had lower LAB counts than uncoated samples.

Enterobacteriaceae counts in the treated groups were significantly ($p<0.05$) lower than the control (Figure 1d). Abdeldaiem et al. (1) did not identify Enterobacteriaceae in coated carp fish fillets with calcium caseinate film containing essential rosemary oil during 12 days of refrigerated storage. In previous studies, nanoemulsions of various plant oils and essential oils reduced TMB and TSB, Enterobacteriaceae, and LAB counts in fish (12, 26), chicken (28), beef (18), and pork (11).

The different nanoemulsions were shown to have antibacterial effects against various microorganisms (18, 13). Nanoemulsions integrate with lipid membranes, destabilizing cytoplasmic membranes, releasing essential oils, and causing cell death in microorganisms (18, 12).

Carvacrol and thymol are responsible for the antibacterial properties of ZEO (4), which are the main constituents of the essential oil. The lipophilic property of the essential oil, especially monoterpene compounds, enables the oil to penetrate the cytoplasmic membrane of the

microorganisms and disrupts its function, thereby causing cell death (10).

Microorganisms' proteolytic enzymes have produced volatile nitrogenous compounds (10, 31, 14). These results align with those of Abdeldaiem et al. (1), who reported that coated carp fillets with calcium caseinate film had low TVN levels during storage.

The phenolic compounds, including carvacrol and thymol, neutralized free radicals and thus reduced lipid oxidation (10, 16). Nanoemulsion formation reduced the droplet size and increased the specific surface area, so radical scavenging occurred faster and more effectively. SPI-MMT+2% ZNE was more effective than other groups in controlling the oxidation of burger samples due to the essential oil's gradual release (10). Hassanzadeh et al. (18) reported decreased TBARS levels in chitosan-coated chicken breasts containing grape seed extract and showed a delay in increasing total volatile nitrogen and lipid oxidation in coated lamb (18).

The activity of microorganisms and the increasing volatile bases can increase the pH values. Similar findings have been reported in the literature (31, 32).

The scores of all sensory properties in all experimental groups decreased significantly during 16 days ($p<0.05$), but it was slower than the control group in the treated groups. These results confirmed those of microbial and chemical analysis. Previous studies have shown that treating different types of meat with different nanoemulsions based on essential oils increased their shelf life during refrigeration (28, 10, 18, 12, 13).

Conclusion

The results showed that soy protein isolate-montmorillonite nanoclays containing ZEO nanoemulsion effectively controlled the population of microbial flora and delayed physicochemical changes in chicken burgers. In this regard, SPI-MMT+2% ZNE was the most influential group for increasing the shelf life of chicken burgers. As a result, the nanocomposite film containing nanoemulsions of ZEO may be suggested to preserve chicken burgers during chilled storage.

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Conflict of Interest

The authors declare the existence of any conflict of interest in this study.

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Investigation of the Effects of Blueberry Powder on the Ripening of Turkish White Cheese

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ABSTRACT

This study aims to enrich Turkish white cheese with blueberry powder, owing to its exceptional bioactive properties, to enhance its functionality. Five cheese samples were prepared by adding different concentrations [0 (control), 0.5, 1, 1.5, 2%] of blueberry powder to the cheese curd. The cheese samples were ripened in vacuum packages for 90 days at 7±1°C. The pH, dry matter, salt, fat, total protein, titration acidity, water-soluble nitrogen, ripening index, electrophoretic casein fractions, color and sensory analyses were performed on the 3rd, 30th, 60th, and 90th days of ripening period. The data obtained were compared in terms of cheese types and ripening times. The addition of blueberry powder to cheese curd and the storage time affected the pH values significantly ($p<0.05$). Similarly, the addition of blueberry increased the titratable acidity values of white cheese and the differences in acidity between cheese samples were found to be significant ($p<0.05$). The highest decrease in the amount of α_{s1}-casein was recorded in C2 (1% blueberry added cheese) samples and the least decrease was in the control group cheeses. Color analysis indicated that the *L* value was reduced with increasing concentrations of blueberry addition because of darkening. In conclusion, blueberry added Turkish white cheese could be produced as an alternative dairy product with acceptable sensory properties.

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Introduction

Turkish White cheese is a brined (or pickled) cheese variety with a soft or semi-hard texture and a salty, acidic taste. Considering its texture, taste and production methods, Turkish White cheese is comparable to some other cheese varieties such as Feta and Domiati (1). Some aspects of this cheese are reviewed, including milk supply, use of starters and enzymes, manufacturing technology, chemical composition and microflora, chemical and biochemical changes during ripening. These cheeses are generally rich in proteins and some minerals however, their bioactive properties are limited. Therefore, it is important to improve its bioactivity (i.e., antimicrobial activity, antioxidant activity, phenolic content) and functionality using ingredients with high bioactivity. A variety of ingredients including nuts, almonds, walnuts, peanuts, nuts, cereals, sugar, and sugary products, fruits and vegetables and their juice, concentrate, puree, paste, products such as honey, cocoa, coffee, chocolate, spices edible parts of plants have been added to cheese. When 10% pineapple is added to Queso de pina cheese, the resulting composition is 60.77% moisture, 19.26% fat, 12.88% total protein, 1.46% salt and pH 6.34 (2). Choi et al. (3) reported that pH values were between 5.24 and 5.39, and fat values between 31.72% and 33.52%, as a result of their study by adding fruit flavors to Gouda type cheeses. Yerlikaya and Karagözlü (4) found that the addition of caper fruit to cheese caused significant improvements in terms of salt, lactic acid, and mineral substances, in line with their analysis of some physicochemical and functional properties of white cheese with caper fruit. It has also been determined that the addition of caper fruit has a positive effect on some physicochemical properties of cheese and differentiates some quality properties. Da Silva et al. (5) investigated the antimicrobial activity of extracts obtained from dried fruit and leaves of blueberry (*Vaccinium corymbosum*) and discovered that leaf extracts were more effective than fruit extracts. In this research, whether the extract of blueberry fruit is dry or wet, it was determined that it has a very good antimicrobial activity regardless of fruit or leaf.

Blueberry is a medicinal plant that is widely used against many health problems. Blueberry are

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used in a variety of food products, particularly in Europe and America, including cake, jam, molasses, marmalade, fruit juice, ice cream, fruit yoghurt, fruit muffins, and wine. Blueberry leaves are used to make tea, and its roots, fruits, flowers, and leaves are used in the production of medicine. It is known with different names such as tea currant, cranberry, and blueberry in different locations. Blueberries have a high-water content (6) hence prone to microbial spoilage and chemical degradation. Therefore, it is sometimes used in dried and ground form, for instance, as a sweetener for diabetics. However, traditional drying methods may harm its bioactive and physical properties due to heat treatment. Freeze-drying can be used as an alternative method to maintain its bioactive properties and color, but it is an expensive method with high-cost setup. As a solution, the development of a method that is both inexpensive and do not damage the components is being considered. One way is the spray drying method which relatively affordable however, due to the high temperature, this method may produce undesirable results (7). In this regard, vacuum drying may offer an efficient method for drying of blueberries.

Fruit and fruit-flavored cheese varieties can provide both the nutritional value of cheese and bioactive properties of the fruit that is contained. Hence, they could present a healthy option for the consumers. The aim of this study is to add blueberry powder dried by vacuum oven, to preserve the bioactive components and maintain the color, into cheese to increase the bioactvitiy and functionality of cheese. Also, it was aimed to develop an alternative cheese with fruity flavor and aroma with an attracting appearance for consumers that avoid eating cheese due to its odor and taste.

Materials and Methods

The white cheese used in this study; was produced in a laboratory environment. The fresh cow milk (4% fat, pH of 6.5, and dry matter of 13.12%) was obtained from Bulancak district of Giresun province was used in the production of cheese samples. The commercial rennet obtained from Intermak Makina product Inc. (8000 mcu/ml) was used as coagulant. Blueberries were collected form Giresun plateaus, and after they were slightly crushed, they were spread on plates in thin layers and dried in a vacuum oven (50°C, 24 h) and ground into powder using a coffee grinder. The blueberry powder concentrations were determined based on preliminary sensory trials. Following production, cheese samples were packed using a vacuum packing machine (Cas Cvp-260, Czech Republic). The packaging material is made of 360 µm thick polyethylene plastic, which is suitable for the product and has very low oxygen and odor permeability.

Cheesemaking

The cow milk was pasteurized at 75°C for 30 s and cooled to 34°C for rennet addition. Rennet was added (3 mL/15 L milk) and kept for 90 minutes to reach cutting maturity. Then, the curd was cut, and whey was removed. Blueberry powder was added to the curd [0% (CC), 0.5 % (C1), 1.0 % (C2), 1.5 % (C3), and 2.0 % (C4)] and homogenized before pressing the curd. After the addition of blueberries, the curds were left in the press overnight. Following the completion of the pressing process, the samples were removed from the pressing cloth and salted with 4% salt (w/w %). To ensure that the salt spreads homogeneously during the salting process, each surface was salted separately. The cheese samples were vacuum-packed and ripened at 7±1°C for 90 days. Two replicates of cheese samples were prepared for each cheese type.

Chemical and Biochemical Analysis

To determine the dry matter content, the cheese samples were dried in a laboratory oven at 105 °C until a constant weight was obtained. The total nitrogen (VELP Scientifica, Italy) concentration of the samples was determined according to Kurt et al. (8). Fat content was determined by the Gerber method. Salt content was determined according to the Mohr method, while pH was measured with a digital pH meter (Starter 3100, USA) as described by Case et al. (9). Water-soluble nitrogen (WSN) and ripening index (WSN/TN) values were calculated using methods developed by Kamaly et al. (10) and Butikofer et al. (11), respectively. Electrophoretic analysis of protein patterns was performed by the method of Celik and Tarakci (12), as previously described by Creamer (13), with some modifications.

Color Analysis

Color measurements were performed using a colorimeter (Minolta Chroma Meter, CR-400, and Osaka, Japan). The *L**, *a**, and *b** color measurements were determined according to the

CIE Lab color system. Three readings were taken for each sample and arithmetic means were calculated.

Sensory Analysis

Sensory evaluation of blueberry-added white cheese was performed by a panel of ten semi-trained graduate students experienced in the sensory evaluation of cheeses. Before evaluation, each cheese was cut into 20 g cubes, left at room temperature (25°C) for 2 hours, and randomly served to the panelists. Overall sensory quality was assessed using a hedonic scale method (1-10 points), with 1 being unacceptable and 10 being very good for color and appearance, smell, structure and texture, taste, and flavor. The panelists were given a glass of water to rinse their mouths between cheese samples. Panelists were also asked to report any flaws in color and appearance, texture, odor, taste, and overall acceptability.

Statistical Analysis

All analyses were performed in duplicate. Minitab 16.0 Statistical Software (Minitab Inc.) was used for all statistical calculations, and the results are presented as mean ± standard deviation. Analysis of variance (ANOVA) was used to determine significance, followed by Tukey's multiple range tests. The significance level of $p<0.05$ was used for statistical differences.

Results and Discussion

Chemical Analysis Results

Table 1 shows the results of the chemical values of the cheeses produced with blueberry addition. The lowest dry matter value was determined with 43.82±0.71% in the C2 sample on the 30th day of ripening; and the highest dry matter value was found in the C3 sample on the 30th day of ripening with the rate of 47.68±0.85%. The effect of cheese type on dry matter values was found to be statistically significant ($p<0.05$), but no significant ($p>0.05$) increase was observed in dry matter values during the ripening period. This could be explained by the blueberry powder being added to the samples at different rates. Similar values were obtained by Davide et al. (2) in queso de pina cheeses with pineapple, Uraz and Şimşek (14) in white cheeses, Yerlikaya and Karagözlü (4) in white cheeses with caper, and Sağun et al. (15) in the brined herb cheese. The effect of blueberry powder on the fat content of cheeses was significant ($p<0.05$), while the differences between ripening periods were insignificant ($p>0.05$). The lowest fat rate was determined as 24.00±0.50% in 2% blueberry cheese on the 3rd day of ripening. During ripening, except C2, a decrease in fat content was observed.

Table 1. Changes in dry matter, fat, titratable acidity, pH, salt and ash content values during the ripening of cheese samples

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
Dry Matter (%)	CC	44.59±0.74^{a,B}	45.39±0.00^{a,B}	45.78±0.04^{a,A}	44.61±1.46^{a,B}
	C1	45.72±0.75^{a,B}	44.47±0.79^{a,B}	46.23±0.47^{a,A}	44.00±1.08^{a,B}
	C2	45.24±0.21^{a,B}	43.82±0.71^{a,B}	45.83±0.98^{a,A}	46.35±0.22^{a,A}
	C3	44.92±0.57^{a,B}	47.68±0.85^{a,A}	46.87±0.41^{a,A}	46.94±0.11^{a,A}
	C4	44.82±0.21^{a,B}	45.30±0.38^{a,B}	45.37±1.12^{a,B}	44.96±0.83^{a,B}
Fat (%)	CC	25.75±0.35^{a,A}	25.50±0.00^{a,A}	25.50±0.00^{b,A}	25.25±0.35^{a,A}
	C1	25.75±0.60^{b,A}	25.00±0.50^{a,A}	25.25±0.50^{b,A}	24.00±0.55^{b,A}
	C2	24.00±0.50^{b,A}	25.00±0.50^{a,A}	26.00±0.50^{a,A}	25.00±0.55^{a,A}
	C3	25.25±0.35^{a,A}	25.00±0.50^{a,A}	26.25±0.50^{a,A}	25.25±0.35^{a,A}
	C4	25.50±0.35^{a,A}	25.00±0.41^{a,A}	24.00±0.41^{b,A}	24.50±0.35^{a,A}
Titratable acidity (Lactic acid, %)	CC	0.40±0.06^{cd,C}	0.69±0.01^{cd,B}	0.82±0.01^{c,A}	0.85±0.06^{cd,A}
	C1	0.39±0.01^{a,C}	0.82±0.02^{ab,B}	0.94±0.02^{ab,A}	1.10±0.03^{a,A}
	C2	0.40±0.03^{ab,C}	0.85±0.06^{a,B}	0.90±0.04^{b,A}	0.95±0.06^{ab,A}
	C3	0.40±0.03^{bc,C}	0.72±0.03^{bc,B}	1.00±0.06^{a,A}	0.88±0.05^{bc,A}
	C4	0.45±0.04^{a,C}	0.70±0.06^{d,B}	0.73±0.01^{d,A}	0.75±0.04^{d,A}
pH	CC	4.73±0.02^{b,A}	4.77±0.01^{b,B}	4.81±0.00^{b,B}	4.79±0.01^{b,B}

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
	C1	6.48±0.03^{a,A}	5.48±0.01^{a,B}	5.52±0.01^{a,B}	5.42±0.02^{a,B}
	C2	5.93±0.02^{a,A}	5.41±0.02^{a,B}	5.49±0.03^{a,B}	5.64±0.02^{a,B}
	C3	635±0.07^{a,A}	5.68±0.04^{a,B}	5.43±0.01^{a,B}	5.51±0.01^{a,B}
	C4	4.68±0.02^{b,A}	4.60±0.01^{b,B}	4.68±0.01^{b,B}	4.62±0.00^{b,B}
Salt (%)	CC	2.44±0.11^{a,A}	2.92±0.08^{a,B}	2.83±0.04^{a,B}	2.77±0.04^{a,B}
	C1	2.81±0.71^{a,A}	2.39±0.00^{a,B}	2.60±0.04^{a,B}	2.46±0.33^{a,B}
	C2	3.63±0.38^{a,A}	2.69±0.00^{a,B}	3.06±0.11^{a,B}	2.19±0.04^{a,B}
	C3	3.31±0.11^{a,A}	2.36±0.12^{a,B}	2.60±0.04^{a,B}	2.51±0.00^{a,B}
	C4	3.36±0.33^{a,A}	2.66±0.87^{a,B}	2.39±0.00^{a,B}	2.34±0.08^{a,B}
Ash (%)	CC	3.27±0.18^{b,A}	3.61±0.06^{b,C}	3.31±0.03^{b,C}	3.18±0.08^{b,B}
	C1	4.03±0.06^{a,A}	3.70±0.02^{a,C}	3.99±0.07^{a,C}	3.96±0.01^{a,B}
	C2	4.67±0.07^{a,A}	3.32±0.09^{a,C}	3.28±0.04^{a,C}	4.23±0.00^{a,B}
	C3	3.96±0.04^{a,A}	3.53±0.13^{a,C}	3.89±0.02^{a,C}	4.00±0.02^{a,B}
	C4	2.92±0.16^{c,A}	3.24±0.06^{c,C}	3.78±0.03^{c,C}	3.75±0.01^{c,B}

a–d indicate differences ($p<0.05$) between columns.
A–C indicate differences ($p<0.05$) between rows.
Mean values ± standard deviation of two trials.

Cheese is a fermented dairy product hence, controlled production of lactic and other acids from lactose by lactic acid bacteria is an essential step during the manufacturing and ripening. Titratable acidity in cheese is composed of lactic acid, formic acid, acetic acid, butyric acid (a lactose fermentation product), free fatty acids formed by lipolysis, and free amino acids formed by proteolysis. The differences between the samples and storage time were found to be significant ($p<0.05$). The acidity of the cheese increases over time due to the high acidity of the fruit added to the cheese. The results obtained are higher than the titration acidity values of Tarakçı and Küçüköner (16) herb-added cheese sample, and Uraz and Şimşek (14) White cheese sample.

The effect of storage time on the pH data of cheese samples was found significant ($p<0.05$). The highest value was detected in the C1 sample on the 3rd day, and the lowest was in the C3 sample on the 30th day. The pH values of the CC and C4 samples are slightly lower than the other samples. Tarakçı et al. (17) herby cheeses, Da Silva et al. (18) fruit added cheese samples, Çakır-Yılmaz (19) spice added cheese samples were found to have similar pH values.

The effect of cheese type on salt content was found statistically significant ($p<0.05$). On the 90th day of the storage period, a decrease in salt values was observed in general. In the dry salting method, the cheese absorbs the salt over time. Therefore, as the storage time increases, the salt value decreases. Salt values of this study are comparable to those found in the Van herby cheese study by Tunçtürk et al. (20), freshly produced circassian cheese samples by Uysal et al. (21), and local herb-added cheeses study by Agboola and Radovanovic-Tesic (22). Davide et al. (2) added pineapple to queso de pina cheeses and determined a higher salt content. Ash rates were found to be between 2.75% and 4.67%. The effect of cheese type on the ash concentrations was found significant ($p<0.05$). The change in ash rates were found to be similar to the changes in salt rates.

Changes in the Protein, WSN, WSN/TN of Cheeses during Ripening

Cheese texture is formed by casein-casein, casein-water, and casein-fat interfaces, state of ionic or bound (to the casein matrix) calcium, state of bulk or bound (to casein) water, and the degree of proteolysis. The distribution and binding capability of water affect the structure, for example, the casein matrix becomes porous and tortuous (23). It was determined that the protein ratios of white cheeses produced with different amounts of blueberry fruit were between 14.88% and 17.69%. The results suggested that the fruit added to the cheese did not affect the protein values. Agboola and Radovanovic-Tesic (22) herb cheese, Tarakçı et al. (24) herbed cheese, Tunçtürk et al. (20) in kashar cheese, Tarakçı and Deveci (25) in spicy

white cheese, Davide et al. (2) queso de pina cheese with pineapple and Yerlikaya and Karagözlü (4) cheese with capers determined similar ripening values. One method for determining the rate of proteolysis in cheeses is to measure the rate of water-soluble nitrogen (WSN). It has been reported that the acidity in cheese is primarily a result of lactic acid, acetic acid, butyric acid, formic acid however, free amino acids, alkaline and neutral compounds formed by proteolysis, as well as lipolysis degradation products, can cause a decrease in titratable acidity (26, 27).

Water soluble nitrogen (WSN) amount is a ripening parameter (28). It has been determined that the water-soluble nitrogen ratios of the cheeses were between 0.17-0.51%. Statistical differences between samples were found to be significant ($p<0.05$). This deviation is estimated to be due to errors that occurred during the instrumental analysis.

The ripening index is calculated by proportioning the total WSN to the total nitrogen amount. According to the results obtained from the study, it was noted that the differences between the samples were significant ($p<0.05$). According to the data given in the Table 2, the highest degree of ripening value was observed on the 90th day for the C1 sample, and the lowest degree of ripening value was observed on the 3rd day for the C4 sample. The ripening index results of the cheese samples obtained were similar to those of Gezmiş and Tarakçı (29) spice-added circassian cheese, Koçak et al. (30) kashar cheese samples, and Tarakçı and Küçüköner (16) herb cheese samples.

Table 2. Changes in the protein, WSN, WSN/TN during the ripening of white cheeses

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
Protein (%)	CC	15.18±0.13^{a,B}	15.72±0.38^{a,B}	15.90±0.13^{a,AB}	15.81±0.25^{a,A}
	C1	15.63±0.76^{a,B}	15.56±0.00^{a,B}	16.61±0.38^{a,AB}	15.99±0.50^{a,A}
	C2	15.99±0.00^{a,B}	14.92±0.51^{a,B}	15.72±0.63^{a,AB}	16.52±1.26^{a,A}
	C3	14.88±0.71^{a,B}	15.99±0.51^{a,B}	15.54±0.13^{a,AB}	17.69±0.63^{a,A}
	C4	15.98±0.24^{a,B}	16.17±0.51^{a,B}	17.40±0.38^{a,AB}	16.45±0.00^{a,A}
WSN (%)	CC	0.19±0.00^{b,C}	0.28±0.01^{b,B}	0.35±0.01^{b,A}	0.31±0.01^{b,A}
	C1	0.19±0.00^{b,C}	0.40±0.00^{a,B}	0.49±0.02^{a,A}	0.51±0.01^{a,A}
	C2	0.21±0.01^{a,C}	0.37±0.00^{a,B}	0.44±0.01^{a,A}	0.41±0.02^{a,A}
	C3	0.20±0.00^{a,C}	0.37±0.00^{a,B}	0.47±0.01^{a,A}	0.50±0.00^{a,A}
	C4	0.17±0.01^{c,C}	0.20±0.00^{c,B}	0.26±0.01^{c,A}	0.27±0.00^{c,A}
WSN/TN (%)	CC	7.90±0.03^{c,C}	11.46±0.01^{d,B}	14.18±0.09^{d,A}	12.62±0.10^{d,A}
	C1	7.64±0.39^{d,C}	17.40±0.00^{a,B}	18.93±1.16^{b,A}	20.23±0.87^{a,A}
	C2	8.20±0.37^{b,C}	15.83±0.54^{c,B}	18.04±0.52^{c,A}	15.67±0.61^{c,A}
	C3	8.69±0.19^{a,C}	14.73±0.47^{b,B}	19.33±0.59^{a,A}	18.06±0.81^{b,A}
	C4	6.75±0.36^{e,C}	7.75±0.21^{e,B}	8.92±0.70^{e,A}	11.12±0.02^{e,A}

a–d indicate differences ($p<0.05$) between rows.
A–C indicate differences ($p<0.05$) between columns.
Mean values ± standard deviation of two trials.

Casein Fractions in Cheese Samples

Proteins in cheese are broken down by proteolytic and other degrading enzymes. As a result, large and small peptides, amino acids, and smaller organic molecules are formed and this hydrolysis is monitored by different methods (31). One of these methods is the gel electrophoresis method, which detects coarse peptides. At the same time, gel electrophoresis method has been seen as a suitable method for tracking straight chains in casein micelles in the early stages of cheese ripening (32). The images of the gel electrophoresis determination showing the ripening time of the cheeses produced by the Urea-PAGE electrophoresis method are given in Figure 1.

Electrophoretic properties of the blueberry cheese samples and the casein fractions of the control cheese sample were observed in the bands on the 3rd, 30th, 60th, and 90th days of ripening, respectively. When the casein fractions in the gels are examined, it can be seen from the

figures that β-casein and αs_1-casein densities decrease during the ripening period. The highest decrease in the amount of α_{S1}-casein was recorded for C2 (1% blueberry added) cheeses and the least decrease was seen in the control group cheeses. Considering β-casein, the C2 (1% blueberry added) cheese sample again showed the highest decrease, and the cheeses from the control group showed the least decrease. In general, the lowest values were found on the 3rd day and the highest values were found in the cheese samples at 90th day. The differences between the samples were found to be significant ($p<0.05$). Similarly, Gezmiş and Tarakci (29) spice-added circassian cheese, Tarakçı et al. (23) herbed cheese, and Tunçtürk et al. (20) herbed cheese, Tarakçı and Deveci (25) spice-added white cheese samples determined a decrease in the ratios of α_{S1}-casein and β-casein throughout the period.

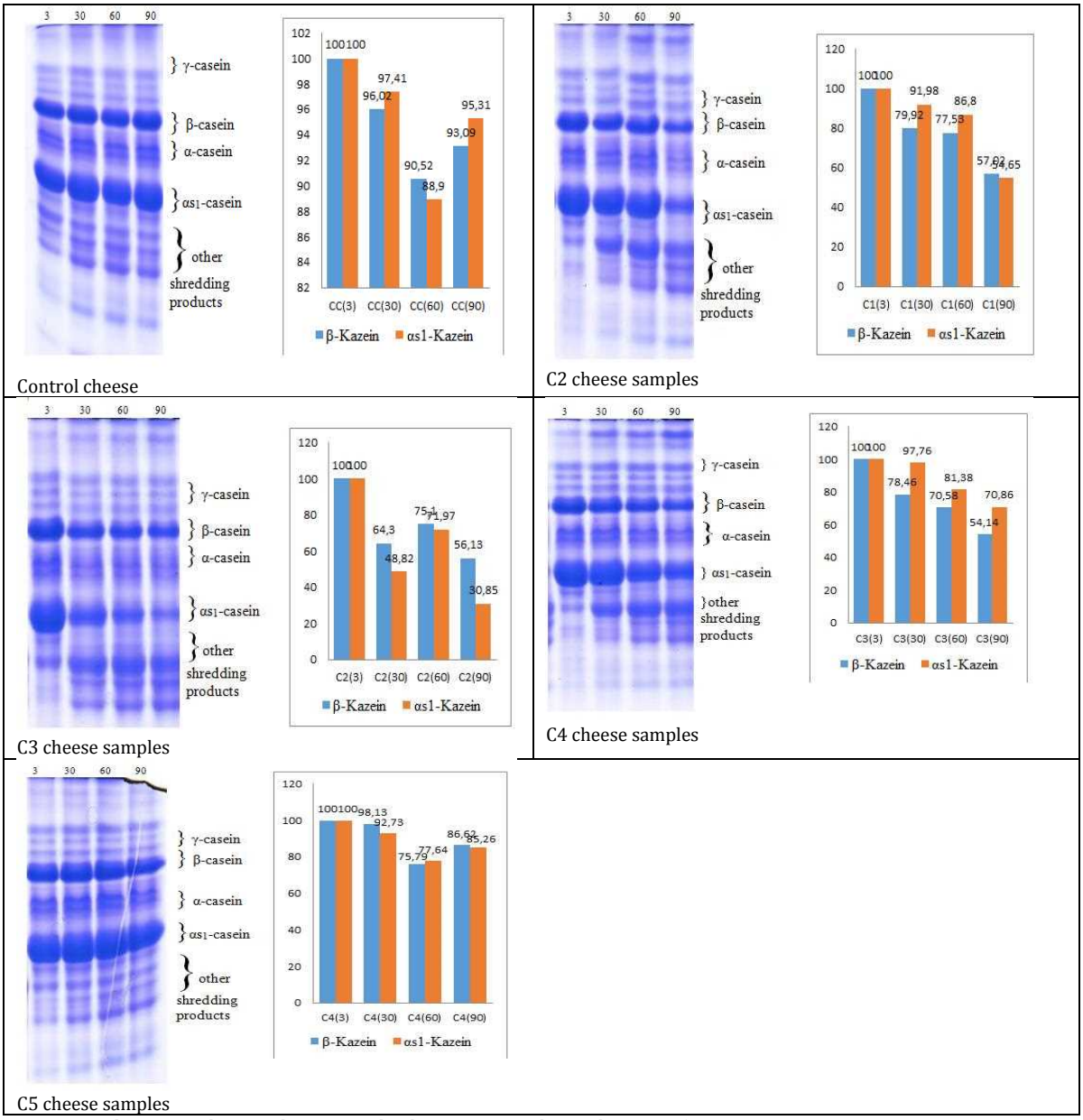

Figure 1. The images showing the ripening by the Urea-PAGE electrophoresis. C5?

Changes in the Color Values of Cheese Samples

L, *a*, and *b* color data are represented by a three-dimensional coordinate system. In this system, the *L* color value represents the color tone going from brightness (100) to darkness (0) on the vertical axis, while +a refers to red, -a to green, +b to yellow, and -b to blue. The *L* color value of the white cheese samples we produced is presented in Table 3. It has been determined that *L* color values are between 68.84 and 92.50. In line with the data we obtained, the differences between the samples were found to be significant ($p<0.05$). The *L* value was higher in the control group samples than in the blueberry cheese samples. As the fruit ratio of cheese increases, the *L* value decreases due to the dark purple color of blueberry. When the data in the table were examined, it was found that the C4 samples in 60th and 90th days had the lowest *L* values, and the CC cheese samples of 3rd day had the highest *L* value. The high deviation values in some of the measurements are estimated to be due to errors that occurred during the instrumental analysis. The results we obtained are similar to the values in the studies of Çakır-Yılmaz (19) on spice-added kashar cheese, Gezmiş and Tarakçı (29) on traditional spicy circassian cheese, and Tarakçı and Bayram (33) on fruit powder-added kashar cheese while Aydın and Tarakçı (34) determined higher *L* values for the kashar cheese with dried herbs.

Table 3. Changes color values during the ripening of white cheeses

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
L color value	CC	$92.50\pm0.66^{a,A}$	$91.60\pm0.24^{a,A}$	$91.56\pm0.11^{a,A}$	$90.62\pm0.57^{a,A}$
	C1	$84.50\pm3.27^{b,A}$	$83.29\pm0.50^{b,A}$	$85.45\pm0.61^{b,A}$	$87.24\pm1.41^{b,A}$
	C2	$77.35\pm0.64^{c,A}$	$75.66\pm4.89^{c,A}$	$77.34\pm1.03^{c,A}$	$81.85\pm7.02^{c,A}$
	C3	$73.65\pm3.35^{d,A}$	$72.68\pm5.07^{d,A}$	$70.54\pm0.31^{d,A}$	$72.68\pm5.07^{d,A}$
	C4	$75.56\pm4.40^{d,A}$	$72.61\pm1.32^{d,A}$	$68.84\pm2.47^{d,A}$	$68.84\pm2.47^{d,A}$
a color value	CC	$-4.04\pm0.02^{e,A}$	$-4.44\pm0.16^{e,A}$	$-4.23\pm0.16^{e,A}$	$-4.63\pm0.15^{e,A}$
	C1	$-1.25\pm0.77^{d,A}$	$-2.73\pm0.01^{d,A}$	$-2.61\pm0.08^{d,A}$	$-2.29\pm0.27^{d,A}$
	C2	$0.46\pm0.52^{c,A}$	$0.13\pm1.33^{c,A}$	$-0.46\pm0.29^{c,A}$	$0.24\pm0.15^{c,A}$
	C3	$0.76\pm0.59^{b,A}$	$1.03\pm0.95^{b,A}$	$1.55\pm0.31^{b,A}$	$1.65\pm0.45^{b,A}$
	C4	$2.59\pm0.26^{a,A}$	$2.83\pm0.37^{a,A}$	$3.34\pm0.22^{a,A}$	$3.63\pm0.30^{a,A}$
b color value	CC	$16.58\pm0.96^{a,B}$	$18.94\pm0.81^{a,A}$	$18.30\pm0.07^{a,A}$	$16.20\pm0.76^{a,B}$
	C1	$10.59\pm0.89^{b,B}$	$16.32\pm0.49^{b,A}$	$16.96\pm0.36^{b,A}$	$11.78\pm0.01^{b,B}$
	C2	$9.08\pm1.21^{c,B}$	$13.61\pm0.70^{c,A}$	$13.28\pm0.96^{c,A}$	$11.78\pm0.01^{c,B}$
	C3	$6.13\pm1.15^{d,B}$	$9.97\pm2.88^{d,A}$	$9.22\pm0.41^{d,A}$	$8.93\pm2.23^{d,B}$
	C4	$6.64\pm1.02^{d,B}$	$9.19\pm1.70^{d,A}$	$8.29\pm0.52^{d,A}$	$7.10\pm1.77^{d,B}$

a–d indicate differences ($p<0.05$) between columns.
A–C indicate differences ($p<0.05$) between rows.
Mean values ± standard deviation of two trials.

It has been determined that *a* color values are between -4.63 and 3.63. It was determined that the differences between the samples were significant ($p<0.05$). The highest and the lowest values were for C4 and C1 samples on the 90th day, respectively. In general, *a* value increased as the amount of fruit powder added to the cheese increased. Based on these findings, it is concluded that adding blueberry fruit to cheese increases its color value. While *a* color value of the cheeses was close to green in the control group samples, the blueberry added cheese samples were more red. The color difference in control cheeses is thought to be due to the milk from which the cheeses are made and the diet of the animal. It is found that the color of blueberry enhances the redness of cheeses. When these results we obtained are compared with other results; Gezmiş and Tarakçı (29) spicy circassian cheese, Tarakçı et al. (24), white cheese, Tarakçı and Deveci (25) spicy white cheese, Tarakçı and Bayram (33) fruity kashar cheese, Çakır-Yılmaz (19) spice added kashar cheese, and Aydın and Tarakçı (34) herb-added kashar cheeses is found to be similar to the values in their studies. While the *b* color values of the cheeses in the control group samples were close to yellow, the values in the blueberry cheese samples were bluer.

Sensory Scores in the Cheese Samples

The sensory scores for the cheese samples during storage are presented in Table 4. The lowest and highest color and appearance scores belonged to C2 sample (5.40) at 90th day and C4 sample (9.20) on the 30th day, respectively. The findings of this study revealed that the differences between the samples were significant ($p<0.05$). Considering color and appearance, C4 sample is found to be the most popular while the least liked cheese was C2. The color and appearance scores got higher as storage proceeded. Similar patterns were observed in the studies by Gezmiş and Tarakçı (29) spicy Circassian cheese, Tarakçı et al. (24) white cheese, Tarakçı and Deveci (25) spicy white cheese, Tarakçı and Bayram (33) fruity kashar cheese.

Table 4. Sensory scores for the cheese samples

	Cheese Types	Ripening Times (Days)			
		3	30	60	90
Color and Appearance	CC	8.10±0.99^{b,A}	8.40±1.58^{b,A}	7.50±1.43^{b,A}	7.40±1.51^{b,A}
	C1	7.00±1.33^{c,A}	6.30±1.06^{c,A}	6.90±1.37^{c,A}	7.00±1.33^{c,A}
	C2	6.20±0.79^{d,A}	5.60±0.97^{d,A}	6.20±0.92^{d,A}	5.40±0.97^{d,A}
	C3	7.00±0.94^{ab,A}	8.70±0.82^{ab,A}	8.40±0.97^{ab,A}	8.80±0.79^{ab,A}
	C4	8.40±1.35^{a,A}	9.20±1.14^{a,A}	8.60±1.08^{a,A}	8.50±1.18^{a,A}
Odor	CC	8.60±0.97^{b,A}	7.00±1.33^{b,A}	7.50±1.43^{b,A}	7.70±1.06^{b,A}
	C1	5.70±1.06^{d,A}	5.50±0.53^{d,A}	5.80±1.03^{d,A}	5.50±0.53^{d,A}
	C2	5.50±0.97^{d,A}	5.50±0.53^{d,A}	5.50±0.71^{d,A}	5.40±0.70^{d,A}
	C3	5.80±1.03^{c,A}	7.20±1.03^{c,A}	6.00±0.82^{c,A}	6.70±1.49^{c,A}
	C4	8.60±1.51^{a,A}	8.60±1.51^{a,A}	9.10±1.20^{a,A}	8.20±1.32^{a,A}
Structure and Texture	CC	9.30±0.82^{b,A}	7.70±1.42^{b,A}	7.90±1.66^{b,A}	8.40±1.65^{b,A}
	C1	6.20±0.92^{d,A}	5.90±0.99^{d,A}	6.10±1.10^{d,A}	6.00±1.16^{d,A}
	C2	5.50±1.08^{d,A}	5.40±0.70^{d,A}	5.50±0.97^{d,A}	5.70±1.25^{d,A}
	C3	6.10±0.99^{c,A}	7.20±0.63^{c,A}	6.60±1.43^{c,A}	7.60±1.43^{c,A}
	C4	9.40±0.70^{a,A}	9.40±1.08^{a,A}	9.50±0.85^{a,A}	9.10±0.99^{a,A}
Taste and Flavor	CC	8.30±1.06^{a,A}	7.40±1.35^{a,A}	6.40±1.65^{a,A}	8.40±0.84^{a,A}
	C1	5.70±1.06^{b,A}	5.70±1.06^{b,A}	6.20±1.23^{b,A}	5.80±1.14^{b,A}
	C2	5.40±0.70^{b,A}	5.40±1.08^{b,A}	5.30±0.48^{b,A}	5.40±1.08^{b,A}
	C3	6.70±1.25^{a,A}	7.40±1.43^{a,A}	8.60±0.84^{a,A}	7.20±1.14^{a,A}
	C4	7.90±1.66^{a,A}	7.00±1.89^{a,A}	7.90±1.10^{a,A}	8.20±0.79^{a,A}
General acceptability	CC	9.00±1.05^{a,A}	7.70±1.34^{a,B}	8.10±1.29^{a,A}	8.20±0.79^{a,A}
	C1	5.40±0.70^{c,B}	5.10±0.57^{c,B}	6.40±0.84^{c,A}	6.60±0.84^{c,A}
	C2	5.70±1.06^{c,B}	5.60±0.84^{c,B}	5.90±0.57^{c,A}	6.10±0.88^{c,A}
	C3	5.50±0.71^{b,B}	7.300±0.95^{b,B}	7.40±0.52^{b,A}	7.80±0.92^{b,A}
	C4	8.50±0.85^{a,A}	8.60±0.84^{a,A}	8.80±0.79^{a,A}	9.00±0.67^{a,A}

a–d indicate differences ($p<0.05$) between columns.
A–C indicate differences ($p<0.05$) between rows.
Mean values ± standard deviation of two trials.

The C4 sample is the most liked and the C2 sample is the least liked regarding odor scores. The odor scores decreased with prolonged ripening. The structure and texture scores were the lowest with 5.40 for the C2 sample on the 30th day, and the highest score with 9.50 for the C4 sample on the 60th day. Considering structure and texture scores, it is observed that the most liked cheese sample is the C4 sample while the least liked one was C2. In general, structure and texture scores decreased over time.

The lowest taste and aroma scores were determined as 5.30 for the C2 sample on the 60th day while the highest score was 8.60 for the C3 sample on the 60th day. In general, the taste and aroma scores increased over time. It was determined that the differences between the taste and aroma scores of the samples were significant ($p<0.05$). Also, it was determined that

the most liked sample was the C3 sample while the least liked one was C2.

Salt, pH, degree of ripening, and cheese composition are improtant factors in the development of cheese flavor and aroma. For this reason, the taste of cheeses produced and ripened differently from each other is also different (35). According to this study, the lowest general acceptability scores were determined as 5.10 for the C1 sample on day 30, and the highest score of 9.0 was for the C4 sample on day 90. When the obtained data are examined, it was observed that the differences between the samples were significant ($p<0.05$). Overall, it was determined that the most liked sample was C4, and the least liked sample was C2. The general acceptability scores showed an increasing pattern over time. Similarly, Tarakçı and Bayram (33) cheddar cheese with fruit addition, Gezmiş and Tarakçı (29) Circassian cheese with spice addition, and Tarakçı and Deveci (25) white cheese with spice addition determined increasing acceptability scores over storage time.

Conclusions

In this study, it was found that organoleptically acceptable white cheese could be produced by adding an optimized concentration of blueberry fruit powder. The addition of blueberry fruit powder affected the chemical, biochemical, and sensory properties of white cheese significantly. According to sensory evaluation and general acceptability data, cheeses with higher blueberry ratio received higher scores. The highest casein degradation was observed in 1% blueberry-added cheeses while the cheeses with 2% blueberry fruit addition were liked the most. Blueberry addition to white cheese improves its taste and offers an alternative product to consumers. This study did not cover the alteration in bioactivity of white cheeses with blueberry powder addition however, future studies should focus on the effect of blueberry addition on the phenolic content and antioxidant activity of white cheese. Also, including the cheeses' organic acid and phenolic profiles would enrich the study.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Cysticercosis in Lamb and Goat Meat and Edible Offal Produced In an Abattoir in Iran in 2021

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ABSTRACT

Introduction: *Cysticercosis* is a worldwide disease that affects farm animals and, in some cases, like bovine and porcine *cysticercosis*, is considered zoonosis. This condition, in sheep and goats, results in economic losses especially due to the condemnation of edible offals or meat. In this concern, the aim of this study was to examine cysticercosis and factors influencing the frequency and weight of relevant meat or red offals condemnation among the sheep and goats slaughtered at a slaughterhouse in Iran.

Methods: A one-year retrospective cross-sectional epidemiological study was carried out to examine the presence of cysticercosis at postmortem inspection. Data regarding the date of slaughter, animal species, sex, and the type of cysticercosis infection (*Cysticercus ovis* or *Cysticercus tenuicollis*) were recorded.

Results: A total of 17530 carcasses were contaminated with different types of cysticercosis, and among them, 9072 offals were rejected and 291 cases were totally condemned. During winter the number of contaminated samples was higher compared to the other seasons. Goats were only infected with *C. ovis* and none of them were totally condemned. The mean proportion of condemned tissues in each contaminated sample was higher in sheep (0.5 kg/case). The damaging effects of cysticercosis in male carcasses were greater than in females, and *C. ovis* infection resulted in higher weight and rate of offal and carcass condemnation.

Conclusion: In conclusion, it seems that a comprehensive antihelminth strategy must be followed by the relevant food animal producers to decrease the economic losses and zoonoses problems caused by cysticercosis.

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Introduction

Red meat has been a significant component of the human diet throughout the evolution of mankind. It is recognized as an important food source of protein and essential nutrients, like iron, zinc, and vitamin B_{12}. In Iran, a main part of red meat is obtained from sheep and goats. These ruminants may harbor different stages of various parasites in their edible organs and tissues (meat and offal). Among those parasites, Taenia spp. are considered important ruminant parasites that cause a significant loss of protein sources during the slaughtering process annually. The disease caused by Taenia spp. is cysticercosis. Humans can also get infected by consumption of undercooked or raw tissues obtained from ruminants harboring immature stages of *Taenia* spp. (1). Sheep and goats are intermediate hosts of several *Taenia* spp. and harbor the larval stages in their organs and tissues. During postmortem inspection at abattoir, the macroscopic lesions of these parasites can be detected making the organ or carcass unfit for human consumption.

Cysticercosis in sheep and goats is caused by the larval or intermediate stage of two important parasites from the dog tapeworm family, *Taenia ovis* and *Taenia hydatigena*. The larval stage of *T. ovis*, known as *Cysticercus ovis*, results in cystic lesions in the skeletal and cardiac muscles of sheep. Over time, the muscular cysts degenerate, calcify, and form small nodules with a gritty texture, known as "sheep measles". On the other hand, *Cysticercus tenuicollis*, the larval stage of *Taenia hydatigena*, migrates through the intermediate host's intestines and can be mainly found in the peritoneal cavity and liver of ruminants (2). The migrating larvae can be found

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primarily in the liver parenchyma within 7 to 10 days and can potentially cause traumatic hepatitis in young animals.

Although cysticercosis in sheep and goats is not classified as a zoonotic disease, it can result in economic losses due to reduced productivity, commercial limitations, and condemnation of organs or whole carcasses at slaughterhouses.

Due to the significant economic losses caused by cysticercosis, particularly in developing countries, this disease has become a major concern in the livestock industry. A major part of these financial damages is related to the condemnation of infected tissues during meat inspection. Monitoring and evaluating the level of condemnation caused by different diseases benefit the food animal industry by having better plans and strategies for controlling farm animal diseases. In this regard, this study was conducted to examine cysticercosis and factors influencing the frequency and weight of relevant condemned meat or red offals in sheep and goats slaughtered in an industrial slaughterhouse in Iran.

Materials and Methods

Study design

The present study was conducted in an industrial slaughterhouse located in Khorasan Razavi province in the east of Iran. The lamb and goat meat and edible offals produced in this slaughterhouse are obtained from 4000-9000 sheep and goats slaughtered per day. A retrospective cross-sectional epidemiological study was carried out from April to March 2021. All of the meat products at this slaughterhouse are examined for the presence of cysticercosis by official veterinary inspectors during postmortem inspection.

Study Animals

The meat products examined in this study were obtained from sheep and goats that brought to the abattoir predominantly from the nearby regions. These animals were kept under traditional and industrial farming systems, and they were transported to the slaughterhouse by different vehicles. The species, date of slaughter, and sex of all of the studied animals were recorded.

Determination of Cysticercosis

For the determination of different types of cysticercosis in the studied products, the classic approach of postmortem examination including observation, palpation, and incision was followed.

The determination of *C. ovis* was carried out by detecting the vesicular larvae encysted in the skeletal or cardiac muscles of the animal. Based on the FAO regulation, typical inspection areas for the determination of cysticercosis include the muscles of mastication, cardiac muscle, triceps, diaphragm and its pillars (3). In the case of heavily infected carcasses, which demonstrated the lesions in at least two of the usual inspection sites, total condemnation was indicated.

Edible offals, including the liver, lung, mesentery, and omentum, and abdominal, thoracic and pelvic cavities were visually examined for detecting *C. tenuicollis*. A transparent cyst filled with watery fluid and a single white scolex with a long neck was considered to be *C. tenuicollis* (2). In mild cases, only the cysts were removed, but if extensive infection was detected the organ, mostly liver, or tissue were condemned.

Statistical analysis

All information obtained during postmortem inspection was stored in a Microsoft Excel spreadsheet (version 2013), and the statistical analysis was carried out by SPSS software (version 16.0). Descriptive statistics were used to measure the frequency and weight of tissue losses in this study. Moreover, the difference between the infection frequency and loss weight among different species, sexes, and seasons were also calculated.

Results

During one year of investigation for cysticercosis, a total of 17530 samples were infected with different types of cysticercosis, and among them 9072 offals were totally rejected and partial condemnation was applied for 8458 cases. Moreover, 291 carcasses were totally condemned due to the heavy cysticercosis infection. In total 8856 kg of infected carcass tissues were condemned during postmortem inspection.

The Impact of the Season

Figure 1 represents the frequency and weight of red offals and carcass condemnation in terms of different seasons of 2021. In this regard, the highest number and weight of condemnation due to different types of cysticercosis was reported in winter with 5728 carcasses and 3298 kg weight of condemnation. On the other hand, during spring, the least frequency and weight of losses

were recorded with values of 2530 animals and 1319 kg respectively. The weights of condemnation in summer and autumn were close together. The highest and lowest proportion of condemnation with the values of 0.57 and 0.4 kg/case were recorded in winter and summer respectively, while no significant difference was recorded in spring and fall.

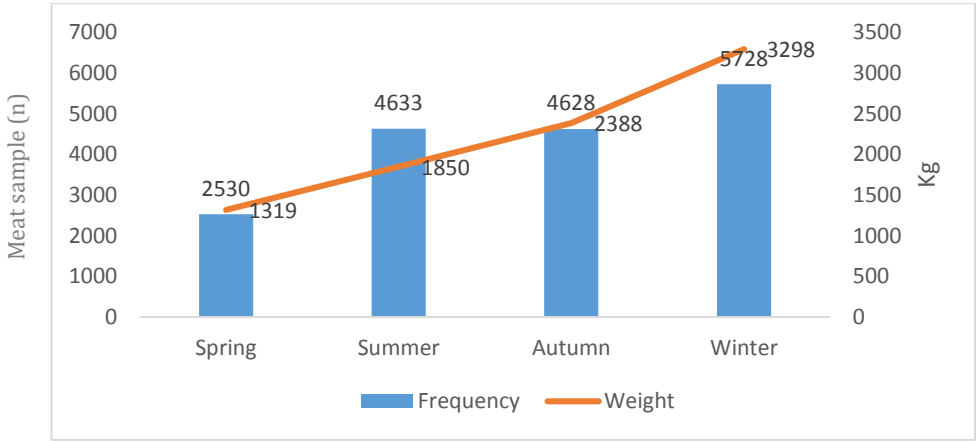

Figure 1. The effect of season on the number and weight of condemnation

Table 1. Differences in judgment and type of condemnation in infected cases in different seasons.

		Season	Frequency (n)	Weight (kg)
Judgment	Partial condemnation	Spring	2483	480
		Summer	2489	848
		Autumn	4547	796
		Winter	5620	1058
	Total condemnation	Spring	47	839
		Summer	55	1002
		Autumn	81	1592
		Winter	108	2240
Type	Trimmed	Spring	1038	202
		Summer	2013	358
		Autumn	2735	448
		Winter	2672	502
	Whole offal	Spring	1492	1117
		Summer	2631	1492
		Autumn	1893	1940
		Winter	3056	2796

Table 1 represents the effects of season on judgment and type of condemnation in contaminated samples. Here the number of total carcass condemnations and number of partial condemnations in winter was greater than in other seasons followed by autumn. However, regarding the type of condemnation, during winter and fall, the number of trimmed cases was close, while whole offal condemnation was also more prevalent in winter.

The Effect of Animal Species

Based on the results, during one year 17520 sheep and 10 goats were infected with different types of cysticercosis corresponding to 8853 and 10 kg of tissue condemnation respectively. Regarding the proportion of tissue losses in each species, sheep with value of 0.5 kg/case demonstrated a higher proportion of condemnation per animal compared to goats with a value of 0.2 kg/case. As shown in Table 2, most of the contaminated sheep or goat carcasses were partially condemned, and only 1.6% of sheep cases was totally condemned. On the other hand, the number of whole edible offal condemnation and trimmed samples were close in sheep. None of the goat cases were totally condemned or had their infected organs trimmed.

Table 2. Differences in judgment and type of condemnation in contaminated cases among different animal species.

		Species	Frequency (n)	Weight (kg)
Judgment	Partial condemnation	Sheep	17229	3180
		Goat	10	2
	Total condemnation	Sheep	291	5673
Type	Trimmed	Sheep	8458	1510
	Whole offal	Sheep	9062	7343
		Goat	10	2

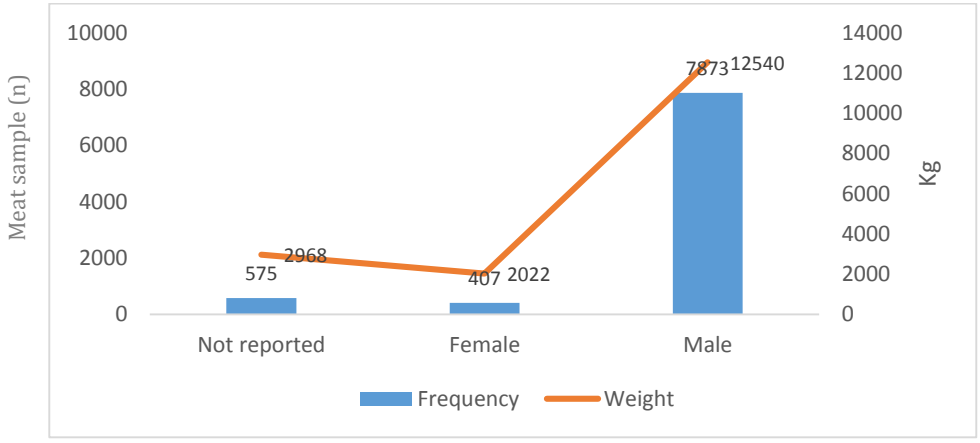

Figure 2. The effect of sex on the number and weight of condemnation

Table 3. Differences in judgment and type of condemnation in infected cases among different sex.

		Sex	Frequency (n)	Weight (kg)
Judgment	Partial condemnation	Female	2021	389
		Male	12250	2118
	Total condemnation	Female	1	18
		Male	290	5655
Type	Trimmed	Female	586	115
		Male	6693	1163
	Whole organ	Female	1436	292
		Male	5847	6710

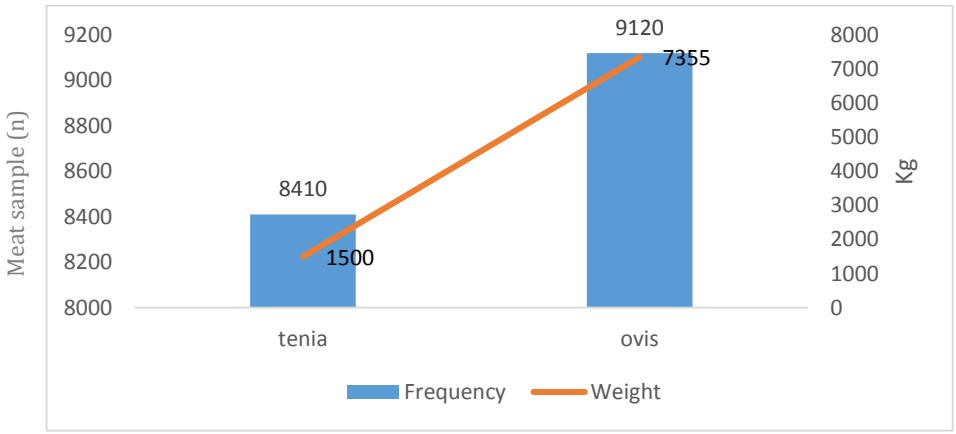

Figure 3. The effect of the type of cyst on the number and weight of condemnation

Sex

In total, 7873 and 407 contaminated male and female cases respectively detected in the studied slaughterhouse during 2021 (Figure 3). It should be mentioned that the sex of 575 cases corresponded to 2968 kg of tissue losses was missing during data collection. The weight of tissue losses in each male carcass was 0.2 kg, which was significantly higher than in female animals (0.62 kg per animal). Table 3 shows that the percentage of male carcasses (2%) which totally condemned during postmortem inspection was much higher than the female cases (0.04%). Almost half of the contaminated offals of male cases were partially trimmed, while total offal condemnation was significantly greater in female cases compared to the trimmed organs.

Table 4. Differences in judgment and type of condemnation in different cases contaminated with different cysts.

		Cyst	Frequency (n)	Weight (kg)
Judgment	Partial condemnation	*C. tenuicollis*	8410	1500
		C. ovis	8829	1682
	Total condemnation	*C. ovis*	291	5673
Type	Trimmed	*C. tenuicollis*	8369	1492
		C. ovis	89	18
	Whole offal	*C. tenuicollis*	41	8
		C. ovis	9031	7337
Species	Sheep	*C. tenuicollis*	84100	1500
		C. ovis	9110	7353
	Goat	*C. ovis*	10	2

Type of Cyst

Data regarding the effects of the type of parasite on frequency and weight of condemnation are presented in Figure 3. As can be seen, while there were no substantial differences in the frequency of infection between *C. ovis* and *C. tenuicollis*, *C. ovis* caused a higher weight of condemnation. In this regard, average tissue condemnation due to the infection by *C. ovis* and *C. tenuicollis* were 0.8 and 0.17 kg/animal respectively. Based on Table 4, all total carcass condemnations were related to *C. ovis*. On the other hand, *C. tenuicollis* caused only 0.4% of total offal condemnation, while almost all (99%) of the offal contaminated with *C. ovis* were totally condemned. Finally, while the number of sheep infected with *C. tenuicollis* or *C. ovis* was close together, goats were only infected with *C. tenuicollis.*

Discussion

The effect of season on parasitic infection in different livestock has been extensively evaluated by several authors. For instance, Hashemnia et al. (2016) reported that the prevalence of ovine cysticercosis in spring (1.8%) was higher than in other seasons, followed by summer, autumn, and winter (4). They stated that the suitable weather conditions in late spring and summer and also the ease of access to acquire infection from contaminated grass led to higher infection rates during warm weather. Hajipour et al. (2020) also reported that the highest prevalence of ovine cysticercosis was in spring, but in summer the lowest rate was recorded (5). In this study, the prevalence of cysticercosis could not be calculated, since the number of total slaughtered animals was unknown. Therefore, the higher number of the infections recorded in winter may be related to the number of animals brought to the slaughterhouse in winter.

Differences between the occurrence of cysticercosis among goats and sheep have been also assessed. Dyab et al. (2017) reported that while both *Cysticercus ovis* and *Cysticercus tenuicollis* were detected in meat products obtained from sheep, goats only harbored *C. tenuicollis* (6). Moreover, they reported a higher prevalence of parasitic lesions in goats. The higher prevalence of *C. ovis* in lamb meat (2.9%) compared to goat meat (1.2%) was reported in another study (5). These differences have been connected to the level of contact between sheep/goats and dogs, and also different protective immunity among them. Unlike the aforementioned studies, in the present study, only *C. ovis* was detected in goat samples. This might be related to the chance and also the lower number of goats slaughtered in the studied abattoir. Moreover, due to the lower number of detected contaminated goat products, it seems irrational to compare the weight and type of condemnation between the two species in the present study.

Regarding the impact of sex on the disease consequences in slaughterhouses, controversial

data existed in the literature. In the present study, the deteriorative effects of cysticercosis in male cases were higher than in females. Mohammed (2021) and Dyab et al. (2017) reported no significant difference in the prevalence of *C. ovis* between males and females at postmortem inspection (6,7). On the contrary, Hashemnia et al. (2016) reported a significantly higher infection rate of *C. ovis* in male sheep compared to females.

The reason why *C. ovis* infection resulted in higher weight and rate of meat and offal condemnation is most probably related to the pathogenesis of the parasite and its pathological lesions on the carcass. The *C. ovis* cysts can generally be generated in skeletal tissues like the cheek, tongue, esophagus, diaphragm, and also cardiac meat. Therefore, the great edible parts of small ruminant carcasses can be condemned during postmortem inspection, while *C. tenuicollis* lesions are generally limited to the surface of the liver or peritoneum, omentum, mesentery and urinary bladder (8), which in comparison are less important tissues of carcasses and their contamination usually results in limited condemnation.

The prevalence of *C. ovis* and *C. tenuicollis* were previously evaluated in different geographical locations. For example, Sissay et al. (2008) reported that in eastern Ethiopia, in sheep meat and edible offal the overall prevalence was 26% for *C. ovis*, and 79% for *C. tenuicollis*, while for goats, the corresponding rates were 22% and 53% (9). In fact, *C. tenuicollis* was more prevalent in both species. A study in Egypt also showed that *C. tenuicollis* was more prevalent in small ruminants meat products compared to *C. ovis* (6).

Conclusion

According to the data gathered and analyzed in the present study, a significant number of meat products and red offals produced in the studied abattoir were infected with cysticercosis, and this resulted in large products and economic losses. In conclusion, based on the level of condemnation, it seems that a comprehensive antihelminth strategy must be followed by the relevant food animal sectors to lessen the level of infection in small ruminants.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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JOURNAL OF NUTRITION FASTING AND HEALTH

In vitro Antimicrobial and Antioxidant Properties of Edible Coating Enriched with *Cinnamomum verum* Essential Oil

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ABSTRACT

Food coatings are a promising strategy to improve the safety and shelf life of food products by inhibiting or retarding the growth of harmful microorganisms. The current study assessed the *in vitro* antibacterial and antioxidant characteristics of a coating based on natural ingredients, including whey protein isolate (WPI), nanochitosan (NCH), bacterial nanocellulose (BNC), and cinnamon essential oil (CEO). The in vitro antibacterial assay of the edible coating solution was performed against four food-born pathogens, consisting *Staphylococcus aureus*, *Listeria monocytogenes, Escherichia coli*, and *Salmonella* Typhimurium. The antioxidant potency of the edible coating solution was evaluated by measuring its capability to scavenge free radicals.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the coating decreased as the CEO concentration increased. The most significant difference in MIC and MBC was observed between the pure coating and the essential oil enriched coating group, which had the maximum essential oil concentration (1.5%). For *Salmonella* Typhimurium bacteria, this difference was 20% for MIC and 15% for MBC. For *Escherichia coli*, it was 15% for MIC and 20% for MBC. For *Staphylococcus aureus*, it was 20% for MIC and 20% for MBC. For *Listeria monocytogenes*, it was 15% for MIC and 20% for MBC.

The antibacterial charachtristics of the coating were evaluated using the disc diffusion technique. The results showed that the coating exhibited considerable antibacterial efficacy against all tested pathogens. The coating also exhibited significant antioxidant activity (up to 5.7% more than the control group).

These findings suggest that the coating based on WPI, NCH, BNC, and CEO has potential applications to improve the food safety.

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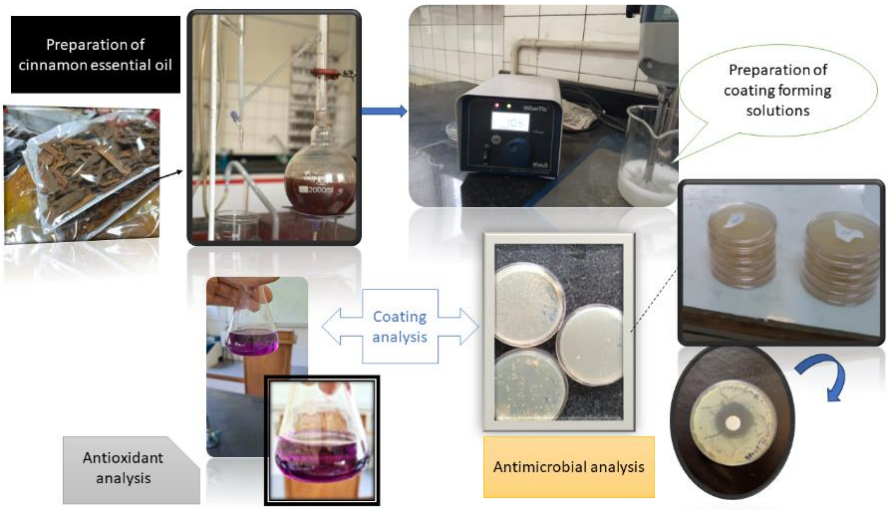
GRAPHICAL ABSTRACT



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Introduction

Food security faces significant obstacles due to the ongoing worldwide population expansion. To meet the needs of future generations, the food supply must be increased. This can be achieved through three main strategies: increasing production, improving distribution, and reducing food waste. One of the most practical strategies to reduce food waste is to protect food samples from microbial, chemical, and oxidative spoilage [1]. Implementing specific methods, such as increased production, improved distribution, and the use of edible coatings, can effectively reduce food spoilage and enhance global food security in the ongoing battle against food loss.

Edible coatings, composed of edible biopolymers derived from food industry wastes or underutilized sources, offer a promising approach for preserving and packaging different food. These coatings improve the shelf life of food products by forming a thin layer on the surface, delaying microbial spoilage, inhibiting moisture loss, and inhibiting lipid, protein, and pigment oxidation [2, 3].

Whey protein isolate (WPI), a group of globular milk proteins, is an excellent matrix for food coatings because of its nutritional and functional properties. The high protein content (90-95%) and minimal fat, lactose, and minerals of WPI make it an ideal barrier against moisture, oxygen, and other gases. Moreover, WPI's emulsifying and foaming properties contribute to uniform and appealing coatings, while its ability to carry active ingredients like antioxidants or probiotics improves the functional properties and nutritional value of food [4].

Nanochitosan (NCH), a derivative of chitosan, a natural, non-toxic biopolymer derived from chitin, plays a crucial role in food coatings due to its unique properties. It possesses superb film-forming capability, antimicrobial and antioxidant activity, and oxygen barrier characteristics, making it an ideal ingredient for extending food shelf life. Furthermore, the capacity of NCH to absorb heavy metal ions decreases food oxidation and improves food safety [5].

Nanocellulose (NC), a biodegradable and renewable nanofiller derived from the breakdown of cellulose fibers, offers a sustainable and versatile alternative for food coatings. Similar to NCH and WPI, NC exhibits remarkable properties that enhance the functionality of food packaging materials. Its abundance, with plants producing around 75 billion tons annually, makes it a readily available resource. NC has three forms: bacterial nanocellulose (BNC), cellulose nanofibrils and cellulose nanocrystals. These nanomaterials have gained traction in packaging applications due to their ability to synergistically enhance the barrier, thermomechanical, and rheological characteristics of nanocomposites. The strong intermolecular and intramolecular hydrogen bonding in NC renders it insoluble in most solvents, contributing to its high strength. nanocellulose is transparent and possesses a reactive surface rich in hydroxyl groups, enabling surface functionalization for diverse applications [6].

In recent years, a paradigm shift has emerged in consumer preferences, favoring natural antioxidants derived from plant and spice extracts over synthetic antioxidants due to concerns regarding the potential toxic effects of the latter. This trend aligns with the growing demand for healthy, chemical-preservative-free foods. Dut to their phenolic content, many herbs and spices exhibit potent antioxidant effects, making them attractive alternatives for food preservation [7].

New approaches to food preservation have been made possible by the growing consumer desire for natural antioxidants and the increasing awareness of essential oils as effective antimicrobials. These strategies provide safer and healthier substitutes for artificial additives. Essential oils (EOs), naturally occurring compounds derived from plants, have gained prominence in food applications related to their remarkable antifungal, antiviral, antioxidant, and antibacterial properties. Cinnamon, a spice gained from the inner bark of Cinnamomum tree species, is particularly noteworthy for its antimicrobial properties. Its essential oil (CEO) possesses two key compounds, cinnamaldehyde and eugenol, which effectively inhibit microbial growth. Moreover, the broad-spectrum antimicrobial activity of cinnamon oil makes it suitable for various food products. These properties position cinnamon oil as a safe and natural alternative to conventional antimicrobial agents [8].

This research aimed to develop and investigate a novel edible coating boosted with the antibacterial and antioxidant characteristics of a mixture of biopolymers and essential oil, as a

healthy and natural alternative to synthetic preservatives.

Materials and Methods

Extraction of CEO

The dried cinnamon bark sample (120 g) was crushed. After that, the ground sample was added into a Clevenger-type apparatus to obtain EO by steam distillation process, as indicated in the European Pharmacopoeia. The collected CEO was dehydrated by sodium sulfate and then kept in opac tubes at four degrees centigrade for further analysis. The components of the collected CEO were identified and mentioned in our previous study [9].

Preparation of WPI/NCH/BNC/CEO Coating

The coating solution consisted of 9 g of WPI powder (w/v), 2 g of NCH (w/v), 1 g of BNC (w/v), and 5 ml glycerol (v/v), which was dissolved in 100 ml distilled water. The solution was maintained at 90°C in a thermostatic bath with continuous agitation for 20 min to promote WPI denaturation and enhance cross-linking among the compounds. After cooling the solution, CEO was incorporated at varying amounts (0.5, 1, and 1.5% (v/v)) and homogenized (Wisd homogenizer, Korea) at 24,000 rpm for two min [10]. One group was prepared without CEO and served as a control (CBW). The other three groups, containing the specified CEO concentrations, were designated as CBW+0.5% CEO, CBW+1% CEO, and CBW+1.5% CEO, respectively.

Microbiological Analysis

In vitro Antibacterial Assay

The antibacterial efficacy of the prepared coatings was evaluated against *Escherichia coli* O157:H7 ATCC 43895 and *Salmonella* Typhimurium ATCC 14028 as Gram-negative bacteria and *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 19117 as Gram-positive bacteria. To prepare inocula, the bacterial cells of each bacterium were transferred to tubes containing 10 ml Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 h. Then, the optical density (OD) of each tested bacterial suspension was adjusted to 0.1 at 600 nm using a spectrophotometer. The resulting cultures (OD 600 nm = 0.1) were diluted in peptone water (0.1% w/v) to obtain a suspension containing about 7 log CFU/ml bacterial cells. The bacterial cell count of the inocula was measured by plating on BHI agar [11].

At first, each bacterial solution (0.2 ml) was added to each Erlenmeyer flask. Next, CEO solutions were made by using tween 80 (Merck, Germany) and distilled water, such that by adding 0.2 ml of each solution to the Erlenmeyer flasks, including liquid culture medium and test bacteria, to prepare concentrations of 0%, 0.015%, 0.03%, 0.045%, 0.09%, and 0.18%. Concentrations higher than this amount were not studied due to the poor solubility of CEO in tween 80 and distilled water. The Erlenmeyer flasks were then incubated in a shaking incubator at 37°C and 140 rpm for 24 h. The minimum inhibitory concentration (MIC) was identified as the lowest concentration of CEO at which there was no visible turbidity after 24 h. The experiment was repeated at lower concentration levels, including 0.01% and 0.005%, to find the MIC if no turbidity appeared at the 0.015% concentration level. After the MIC measurement, the minimum bactericidal concentration (MBC) was identified by transferring 0.1 ml of the of the Erlenmeyer flasks containing no visible turbidity to petri dishes containing the BHI agar medium for each type of bacteria. After 24 h of incubation at 37°C, the bacterial growth was monitored. The first concentration at which no colony was seen was regarded as the MBC [12].

The antimicrobial activity of CBW and CBW+CEO coatings containing 5% DMSO was evaluated by determining the MIC and MBC of coating solutions against the aforementioned foodborne pathogens. Various proportions of coating solutions in BHI broth (5%, 10%, 15%, …50% v/v) of were prepared. After that, prepared solutions were inoculated with 7 log CFU/ml of each tested bacterium and then incubated in a shaking incubator at 37°C for 24 h under continuous shaking (75 rpm). The MIC of coating solutions was defined as the last tube in the dilution series that exhibited no visible turbidity or signs of growth. The MBC of coating solutions was identified by plating 0.1 ml of the contents of each tube without turbidity onto BHI agar and incubating them at 37°C for 24 h [13, 14].

Disc Diffusion Assay

The assessment of antibacterial efficacy was conducted employing the disk diffusion assay as indicated by the clinical and laboratory standards institute's guidelines [15, 16] as follows: 0.1 ml of each bacterial suspension containing

approximately 7 Log CFU/ml was inoculated on the surface of Mueller-Hinton agar plates. Afterward, sterile Whatman paper discs (6.4 mm in diameter) impregnated with 10 µL of coating solutions were put onto the surface of the inoculated Mueller-Hinton agar. Then the plates were incubated at 37°C for 24 h. The diameter of inhibition zones (DIZ) was determined using ImageJ software (version 1.54) [17].

In vitro Antioxidant Activity

The antioxidant potency of the coating solutions was determined by their capability to scavenge DPPH free radicals. In brief, 0.5 mL of various coating solutions were dissolved in 1 mL of methanol and subsequently integrated into 2 mL of a methanolic solution of DPPH (100 mmol/L). The resulting mixtures were agitated and incubated at room temperature in the absence of light for 30 min, following which the absorbance was determined at 517 nm against blank. The antioxidant potency was quantified using the following formula:

Antioxidant activity (%) = [(A_{517} control - A_{517} sample) / A_{517}control] × 100

(A_{517} sample is the absorbance of the mixture of DPPH solution plus coating solution and A_{517} control is the absorbance of pure DPPH solution) [18].

Statistical Analysis

Data analysis was performed utilizing SPSS software (version 27, SPSS, Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was conducted, followed by Tukey's test, to discern any statistically significant variations among the mean values.

Result and Discussion

Chemical Composition of Cinnamon Essential Oil

The dominant constituents of the used CEO were cinnamaldehyde, δ-cadinene, and cis-cinnamaldehyde, respectively. It was documented that cinnamaldehyde is the most abundant component of CEO [19-21].

Other researchers have documented similar compounds; however, notable distinctions exist in concentration levels [22-24]. These variations may be related to divergent environmental factors such as weather conditions, soil composition, seasonal variations, geographic and geological factors influencing the growth of plants, as well as disparities in the age and stage of maturity. Additionally, distinctions may arise from variances in the processing of plant materials before EO extraction and variations in the extraction methods employed [25].

Microbiological Analysis

In vitro Microbiological Analysis

The CEO's MICs and MBCs against the investigated bacterial strains are presented in Table 1. In this study, the MIC and MBC values of CEO against tested bacteria ranged from 0.3 to 0.9 µl/ml and 0.45 to 0.9 µl/ml, respectively. The lowest MIC was observed in *S. aureus* and *L. monocytogenes*, while the highest MIC was recorded in *S.* Typhimurium. Conversely, the lowest MBC value was noted in *S. aureus*, while the highest MBC value was observed in *L. monocytogenes*, *E. coli*, and *S.* Typhimurium.

Table 1. MIC and MBC of CEO

Bacteria	*S.* Typhimurium	*E. coli*	*S. aureus*	*L. monocytogenes*
MIC (µl/ml)	0.9	0.45	0.3	0.3
MBC (µl/ml)	0.9	0.9	0.45	0.9

CEO exhibited intense antibacterial activity against all tested bacterial species. The findings of the current study are in the same boat as those of Prabuseenivasan et al. (2006) and Oulkheir et al. (2017), who also noted that CEO exhibited strong activity against selected bacterial strains [13, 26].

On the other hand, some researches has demonstrated the potent and consistent inhibitory effects of CEO against various pathogens [27]. Several investigations have indicated that the antimicrobial potency of EOs can be related to their characteristic hydrophobic nature and the properties of their components. This hydrophobicity allows essential oils to interact with the lipids of bacterial cell membranes, disrupting cell structures and increasing permeability [28, 29]. The resultant vast leakage from bacterial cells or the release of essential molecules and ions finally results in cell death [30].

The effectiveness of coating solutions in inhibiting four food-borne pathogens is detailed in Table 2. The CBW coating solution exhibited MIC and MBC values ranging from 30% to 35% and 35% to 45%, respectively, against the

following organisms in BHI broth: *S.* Typhimurium (35% MIC and 45% MBC), *E. coli* (30% MIC and 40% MBC), *S. aureus* (30% MIC and 35% MBC), and *L. monocytogenes* (30% MIC and 35% MBC). The incorporation of 0.5% EO with CBW further affected MIC and MBC values for all food-borne pathogens, ranging from 5% to 10%, except MBC values for *L. monocytogenes*, which exhibited no changes. Moreover, at higher concentrations of EO (1% and 1.5%), the MIC and MBC values declined. The impact of EO on MIC and MBC was generally consistent across these organisms, except for the MBC of *S.* Typhimurium and *E. coli* at a concentration of CBW+ 1.5% CEO, which showed no effect on their MBC value when compared to CBW+ 1% CEO. The antimicrobial potency of chitosan is proposed to result from mechanisms such as membrane leakage resulting from interactions between positively charged chitosan and the negatively charged bacterial cell surface, nutrient and essential metal chelation, and chitosan penetration into bacterial cells, thereby hindering DNA transcription [14].

Compared with other EOs, such as Apricot (*Prunus armeniaca*) kernel and *Ferulago angulata* EO, the synergistic effect of CEO on the antimicrobial activities of chitosan was found to be more pronounced [18, 31].

However, nanotechnology's utilization significantly augmented the coating solutions' antibacterial potency, as demonstrated in Table 2. This enhancement was contingent on the specific coating formulation; higher concentrations of CEO were more profoundly affected by nano emulsification. The heightened water dispersibility, coupled with a reduction in droplet size after homogenization, facilitated a more efficient and rapid penetration of antimicrobial constituents via the bacterial cell membrane, thereby amplifying their efficacy [32, 33]. Additionally, the application of severe mechanical stress caused the chitosan molecular chains to break into shorter chains, facilitating easier passage through the bacterial membrane and consequently increasing antibacterial activity [34].

Table 2. MIC and MBC of coatings

Coating Combination	*S.* Typhimurium		*E. coli*		*S. aureus*		*L. monocytogenes*	
	MIC (%)*	MBC (%)	MIC (%)	MBC (%)	MIC (%)	MBC (%)	MIC (%)	MBC (%)
CBW	35	45	30	40	30	35	30	35
CBW+ 0.5% CEO	30	40	25	35	20	30	25	35
CBW+ 1% CEO	20	30	20	20	15	20	20	25
CBW+ 1.5% CEO	15	30	15	20	10	15	15	15

*% of coating solution in BHI broth.

Antibacterial Assessment by Disc Diffusion Assay

The findings of the antibacterial potency of the coating solutions against the tested foodborne pathogen bacteria using the disc diffusion assay are displayed in Table 3. All treatments resulted in the creation of inhibition zones, except for coatings without CEO and CBW containing 0.5% CEO for gram negative bacteria. The integration of higher concentrations of CEO increased the DIZ, and the maximum inhibition zones were determined for CBW containing 1.5% CEO, with average inhibition zone sizes of 16.56, 18.2, 19.22, and 21.14 mm for *S.* Typhimurium, *E. coli*, *L. monocytogenes*, and *S. aureus*, respectively.

Table 3. Inhibition zone (mm)

Coating / Bacteria	CBW	CBW+ 0.5% CEO	CBW+ 1% CEO	CBW+ 1.5% CEO
E. coli	0^{aA*}	0^{aA}	15.9 ± 0.42^{aB}	18.2 ± 0.28^{aC}
L. monocytogenes	0^{aA}	15.34 ± 0.4^{bB}	16.81 ± 0.38^{bC}	19.22 ± 0.31^{bD}
***S.* Typhimurium**	0^{aA}	0^{aA}	15.19 ± 0.3^{aB}	16.56 ± 0.23^{cC}
S. aureus	0^{aA}	16.19 ± 0.44^{bB}	18.37 ± 0.41^{cC}	21.14 ± 0.39^{dD}

*Values are means ± standard deviations. Means with different lowercase letters within the same column are significantly different ($p < 0.05$). Means with different capital letters within the same row are significantly different ($p < 0.05$).

According to the documents, the antibacterial activity of food coatings devolves on some factors, consisting of the type and physicochemical characteristics of integrated antibacterial agents, the composition and concentration of coating materials, and the

technique of preparation of the coating solution [35, 36]. It was documented that flavonoids, aromatics, and esters are the major antibacterial constituents of CEO [37].

Additionally, the antibacterial findings of CBW containing CEO indicate successful incorporation of CEO into the coatings, and its release from the discs immersed with coating solutions during antibacterial tests.

In these cases, a synergistic effect between NCH and BNC is observed. The stiff, slender and rod-shaped particles of BNC have been predicted to damage the bacterial cell membrane seriously. Microbial cells are vulnerable to the cationic effect of chitosan because of this damaged membrane [38-40]. Therefore, these coatings can have good antimicrobial properties even with a small amount of BNC.

In the current research, gram-positive bacteria exhibited greater susceptibility to CBW coatings containing various concentrations of CEO. These findings align with existing literature, which generally suggests that gram-negative bacteria are more resistant to plant EOs (including CEO) than gram-positive bacteria [41].

The chemical composition analysis of CEO suggests that its antibacterial activity is primarily attributed to its high cinnamaldehyde content, a conclusion consistent with previous research [9]. The antimicrobial potency observed was variable and dependent on several factors, including the type of incorporated EO, concentration of EO, microbial group susceptibility, and sample storage time. Higher EO concentrations in the formulations corresponded to increased effectiveness of the coatings. It is noteworthy that, in the context of using EOs in NCH edible coatings, an increase in concentration often determines the difference between the presence or absence of effectiveness.

Furthermore, when combined with essential oils, BNC and WPI exhibit enhanced antimicrobial activity due to synergistic effects. Essential oils, characterized by their volatile and lipophilic nature, can readily penetrate bacterial cell membranes and disrupt internal structures. The tightly packed structure and nanofibrils of BNC further facilitate the penetration of essential oils into bacterial cells (24). Meanwhile, WPI's antimicrobial peptides and enzymes contribute additional antimicrobial activity. Combining BNC, WPI, and essential oils can result in a broad-spectrum antimicrobial effect against a wide range of microorganisms [42, 43].

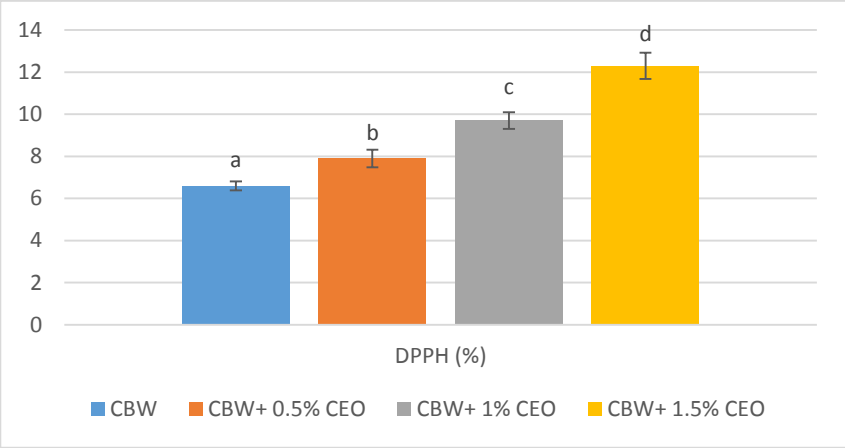

Figure 1. Effect of different concentration of CEO on antioxidant activity of CBW coating
* Different lower case above each graph column indicates significant difference ($p < 0.05$). values are given as mean ± SD.

Antioxidant Activity

Antioxidant activity measurement promotes the production of healthy meals, aligns with consumer health preferences, and provides valuable insights into food quality. In this research, the antioxidant effect of CEO was assessed, and the results are illustrated in Figure 1. Chitosan solution without EO exhibited a 6.6% scavenging activity on DPPH. The antioxidant activity of chitosan increased to 7.9, 9.7, and 12.3 by adding 0.5, 1, and 1.5% CEO, respectively, indicating a concentration-dependent scavenging activity of the chitosan solution on DPPH. The antioxidant potency of CEO may be

related to higher amounts of terpenoid compounds and ample amounts of cinnamaldehyde, which possess high antioxidant activities [44].

Regarding CEO, the results of the current study are consistent with those of Lalami et al., Moarefian et al., and Subki et al., who noted excellent antioxidant activity of CEO [17, 45, 46]. Moreover, the reduction in droplet size of NCH, WPI, BNC, and CEO emulsion after homogenization contributes to a increased specific surface of chitosan-EO, promoting easier and more effectual free radical scavenging. In consistent with these findings, Noori et al. (2018) declared a meaningful increase in the antioxidant potency of sodium caseinate coating enriched with ginger EO through ultrasonic nano emulsification [47].

Furthermore, bacterial nanocellulose (BNC) and whey protein isolate (WPI) exhibit antioxidant activity by scavenging free radicals, protecting cells from oxidative damage, and enhancing the antioxidant activity of other compounds. The hydroxyl groups and hydrogen bonding ability of BNC contribute to its antioxidant properties, while the amino acids and sulfhydryl groups in WPI play a role in its antioxidant activity. Combining BNC and WPI can further enhance antioxidant activity due to synergistic interactions between these materials [48, 49].

Conclusions

The antibacterial assays revealed the susceptibility of four major food-borne pathogens to CBW+ CEO, with this combination exhibiting heightened activity against common food-borne pathogens as the percentage of CEO increased. The addition of higher concentrations of CEO resulted in increased antibacterial efficacy, as evidenced by the expansion of the widths of the inhibition zones. The MIC and MBC of the coating diminished as the concentration of CEO increased, indicating its potential for inhibiting the growth of tested bacteria. Notably, the presented coating demonstrated significant antioxidant effects comparable to the control group, with the capability to scavenge free radicals. These findings underscore the potential of WPI, NCH, BNC and CEO as antibacterial and antioxidant agents. The research acknowledges the necessity of more studies to examine the potential and merits of these components in the food industry. To determine how well the coatings inhibit microbiological growth and food spoilage, these studies should involve evaluating the coatings over an extended period of time on foodstuffs.

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Conflict of Interest

The authors declare no conflict of interest.

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